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Relación de actividades
Programa
SEGURIDADDE LA FISIÓN NUCLEAR
Plan de «Investigación en materia de protección contra las radiaciones»

Beretning
Program
SIKKERHED I FORBINDELSE MED KERNESPALTING
Programmet »Forskning vedrørende Strålingsbeskyttelse«

Tätigkeitsbericht
Programm
SICHERHEIT BEI DER KERNSPALTUNG
Aktion „Strahlenschutzforschung"

Έκθεον πεπραγμένων
Πρόγραμμα
ΑΣΦΑΛΕΙΑ ΣΤΗΝ ΠΥΡΗΝΙΚΗ ΣΧΑΣΗ
Δράση «Έρευνα στον τομέα της Ακτινοσταυίας»

Progress report
NUCLEAR FISSION SAFETY
programme
Action 'Radiation protection research'

Rapport d'activité
Programme
SÛRETÉ DE LA FISSION NUCLÉAIRE
Action «recherche en radioprotection»

Rapporto d'attività
Programma
SICUREZZA DELLA FISSIONE NUCLEARE
Azione «Ricerca sulla radioprotezione»

Verslag van de werkzaamheden
Programma
VEILIGHEID VAN KERNSPLIJTING
Actie „Onderzoek Stralingsbescherming"

Relatório de actividades
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Acção «Investigação no domínio da protecção contra radiações»

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I

EINLEITUNG

INTRODUCTION

INTRODUCTION

DE

VORWORT

In dem vorliegenden Tätigkeitsbericht werden die Ergebnisse der Aktion Strahlenschutzforschung aus dem Zeitraum 1992-1993 wiedergegeben.

Diese Aktion spielt eine grundlegende Rolle in der europäischen Forschung über Strahlenschutz. Sie trägt dazu bei, Zusammenarbeit zwischen den einzelnen Forschungsinstituten und nationalen Einrichtungen zu stimulieren, um die vorhandene wissenschaftliche Kapazität optimal zu nutzen, sowohl im Hinblick auf die begrenzte Anzahl von Wissenschaftlern, als auch auf die zur Verfügung stehenden apparativen und finanziellen Mittel.

Dieser Bericht verdeutlicht den integrierten Charakter der Forschungsvorhaben, die Aktualität der einzelnen Projekte und die praktische Bedeutung der erhaltenen Ergebnisse.

Wie bereits im vorhergehenden Berichtszeitraum (Bericht EUR 14927, 1990-1991) wurden die Forschungsvorhaben als multinationale Mehrpartner Verträge durchgeführt, d.h. an jedem Vertrag beteiligen sich mehrere Institute aus verschiedenen Mitgliedstaaten, um gemeinsam, in koordinierter Weise, ein spezifisches Problem zu untersuchen.

Die Forschungsaktion ermöglichte eine Finanzierung von 76 multinationalen Mehrpartner Vorhaben, die 326 einzelne Forschungsprojekte beinhalten.

Die Tätigkeitsberichte sind nach ihrem Hauptforschungsthema einem der sieben Sektoren zugeordnet worden, die wiederum in drei Haupttägigkeitsbereiche der Strahlenschutzforschung gruppiert wurden:

A) Exposition des Menschen durch Strahlen und Radioaktivität.

   1. Messung der Strahlendosen und ihre Interpretation.
   2. Transfer und Verhalten von Radionukliden in der Umwelt.

B) Folgen der Strahlenexposition des Menschen; ihre Abschätzung, Verhütung und Behandlung:

   1. Stochastische Strahlenwirkungen.
   2. Nicht-stochastische Strahlenwirkungen.
   3. Strahlenwirkungen auf Organismen im Entwicklungtsstadium.

C) Risiken der Strahlenexpositon und ihre Bewältigung.

   1. Abschätzung menschlicher Strahlenexposition und Risiken.
   2. Optimierung und Durchführung des Strahlenschutzes.

In einheitlicher Darstellung geben die einzelnen Tätigkeitsberichte einen Überblick über die in den Verträgen durchgeführten Forschungsarbeiten, gefolgt von den individuellen Berichten der jeweiligen Vertragspartner.

Die Politik der Förderung des Informationsaustausches und der Zusammenarbeit zwischen den Wissenschaftlern ist im Berichtszeitraum mit der Organisation von 19 Studiengruppen, in denen Vertragspartner und andere Fachwissenschaftler zusammenführt werden, sowie von 15 Workshops und Seminaren mit internationaler Beteiligung, verfolgt worden.

Ebenso wurde die koordinierte Ausbildungspolitik im Rahmen der Aktion ERPET (European Radiation Protection Education and Training) fortgesetzt, um einen weiten Fächer von Fachwissen zu erhalten und die Berufschancen junger Wissenschaftler im Strahlenschutz zu verbessern. Sieben Fortbildungslehrgänge wurden im Berichtszeitraum organisiert; sie umfassten: Allgemeinen Strahlenschutz, und spezielle Themenkreise wie strahleninduzierte Mutagenese, Radioökologie, Bewältigung von Notsituationen im Falle eines nuklearen Störfalles, Einsatz und Benutzung von Informationssystemen und Qualitätssicherung während medizinischer, radiodiagnostischer Untersuchungen.

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PREFACE

This volume contains the results obtained during 1992-1993 within the framework of the Radiation Protection Research Action.

This Action plays a fundamental role in European radiation protection research by stimulating collaboration between the various research institutes and national organizations in order to be able to make the best use of the existing scientific capital, from the point of view of the limited manpower, equipment and institutional resources.

This report clearly illustrates the integrated character of this research, the relevance of the subjects covered, as well as demonstrating the practical significance of the progress achieved and results obtained.

As mentioned in the previous report (EUR 14927, 1990-91), the programme is implemented through multinational, multiple partners contracts i.e. each contract involves the participation of several institutes in various Member States to undertake a coordinated investigation of a specific problem together.

The programme supported 76 multinational proposals with multiple partners, covering 326 individual projects.

The progress reports are grouped according to the research topic into one of seven sections falling within the three principal spheres of activity in radiation protection research:

A) Human exposure to radiation and radioactivity:

1. Measure of radiation dose and its interpretation.
2. Transfer and behaviour of the radionuclides in the environment.

B) Consequences of radiation exposure to man; assessment, prevention and treatment:

1. Stochastic effects of radiation.
2. Non-stochastic effects of radiation.
3. Radiation effects on the developing organism.

C) Risks and management of the exposure:

1. Assessment of human exposure and risks.
2. Optimization and management of radiation protection.

As before, each progress report presents an overall picture of the work carried out under a contract with multiple partners, followed by the individual reports of each members of the group.

The policy of promoting cooperation and the exchange of information between the scientists was pursued by organizing 19 study group meetings involving contractors and experts, as well as 15 international seminars and workshops.

In addition, the training policy coordinated under ERPET (European Radiation Protection Education and Training) has been actively pursued to maintain a range of competence and to promote the career prospects for the young research workers in radiation protection. Seven training courses were organized; they cover radiation protection in general, as well as more specialized fields, such as radiation mutagenesis, radioecology, the management of emergency situations created by a nuclear accident and the use of emergency management computer codes, and quality assurance for diagnostic radiology.

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Radiation Protection Research Action

PREFACE

Ce volume contient les résultats obtenus durant l'année 1992-1993 dans le cadre de l'action de recherche en radioprotection.

Cette action joue un rôle fondamental dans la recherche européenne dans le domaine précité en stimulant la collaboration entre les différents instituts et organisations nationaux de recherche, afin de pouvoir tirer le meilleur parti des capacités scientifiques existantes, tant du point de vue du personnel qu'en matière d'équipements et des ressources institutionnelles limitées.

Ce rapport d'activité illustre clairement le caractère intégré de cette action de recherche, l'actualité des sujets traités, ainsi que la signification pratique des progrès réalisés et des résultats obtenus.

Comme précédemment, les contrats sont multinationaux à partenaires multiples, c'est à dire impliquent la participation de plusieurs instituts de différents Etats membres qui, dans le cadre de chaque contrat, étudient conjointement et de façon coordonnée un problème particulier.

Le programme a permis de financer 76 propositions multinationales à partenaires multiples, couvrant 326 projets individuels.

Les rapports d'activité sont regroupés en fonction de l'action de recherche en radioprotection qui couvre trois principaux domaines d'activité:

A) Exposition de l'homme aux rayonnements et à la radioactivité:

   1. Mesure de la dose d'irradiation et son interprétation.
   2. Transfert et comportement des radionucléïdes dans l'environnement.

B) Conséquences de l'exposition de l'homme aux rayonnements; évaluation, prévention et traitement:

   1. Effets stochastiques des rayonnements.
   2. Effets non stochastiques des rayonnements.
   3. Effets des rayonnements sur les organismes en cours de développement.

C) Risques et gestion de l'exposition:

   1. Evaluation de l'exposition de l'homme et des risques.
   2. Optimisation et gestion de la radioprotection.

Cette fois encore, les rapports d'avancement présentent une vue d'ensemble des travaux effectués dans le cadre d'un contrat à partenaires multiples, suivie des rapports individuels de chaque membres du groupe.

La politique de promotion des échanges d'informations et de coopération entre les scientifiques a été poursuivie en organisant 19 réunions de groupes d'étude impliquant des contractants et des experts, ainsi que 15 séminaires et ateliers de dimension internationale.

De même, on a poursuivi la politique de formation coordonnée dans le cadre d'ERPET (European Radiation Protection Education and Training) pour maintenir un éventail de compétences et de promouvoir les perspectives de carrière des jeunes chercheurs en radioprotection. Sept cours de formation ont été organisés; ils couvrent la radioprotection en général, ainsi que des domaines plus spécialisés, tels la mutagénèse radio-induite, la radioécologie, la gestion des situations d'urgence crées par un accident nucléaire et l'utilisation des codes, l'assurance de qualité lors des diagnostiques radiologiques.

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II

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III

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHE EN RADIOPROTECTION

III A

EXPOSITION DES MENSCHEN DURCH STRAHLEN UND RADIOACTIVITÄT

HUMAN EXPOSURE TO RADIATION AND RADIOACTIVITY

EXPOSITION DE L'HOMME AUX RAYONNEMENTS ET À LA RADIOACTIVITÉ

Progress Report

Contract: **F13P-CT920054** **Sector: A1A**

Title: Radiation quantities units and measurement techniques for radiation protection.

1) Allisy ICRU

I. Summary of Project Global Objectives and Achievements

Any determination of human exposure to radiation and radioactivity requires a fundamental set of quantities and units with which exposure c an be specified and the means and ability to make measurements which yield results in terms of these quantities and units. Radiation protection, then, requires the capability to accurately quantify the characteristics and extent of radiation exposure so that appropriate and useful assessments of the potential health consequences and risks can be formulated. The work being carried out via this project seeks to meet these needs.

Important achievements related to these needs include the publication of four new ICRU Reports, the completion of all but the printing of three other ICRU Reports, completion of ICRU review of two draft reports and the completion of major drafting tasks on two other reports.

Head of project 1: Prof. Allisy

II. Objectives for the reporting period

Current Reporting Period

- Publication of reports on (1) interaction data, (2) measurement of dose equivalent, (3) phantoms and (4) stopping powers for protons and alpha particles.

- Preparation of printer's manuscripts for reports on (1) quantities and units for radiation protection, (2) fundamentals of particle counting and (3) specification of therapy doses.

- Completion of major portions of drafting work on reports on (1) conversion factors, (2) *in situ* gamma spectrometry, (3) beta ray measurement for radiation protection.

- Revision of draft report on performance assessment in diagnostic modalities on the basis of ICRU review.

- Initiation of new work on assessment of deposition of radionuclides in the human body.

Next Reporting Period

- Publication of reports on (1) quantities and units for radiation protection, (2) fundamentals of particle counting, (3) specification of therapy doses and (4) *in situ* gamma spectrometry.

- Preparation of printer's manuscript for a report on performance assessment in diagnostic modalities.

- Completion of drafting work on reports on (1) conversions factors for translation of calibration quantities to limiting quantities, and (2) beta ray measurement for radiation protection.

- Initiation of new work on (1) assessment of image quality in mammography, including consideration of dose to the patient, and (2) dosimetric procedures in diagnostic radiology.

III. Progress achieved including publications

Fundamental to radiation protection is a set of dose equivalent quantities, units and concepts that can be applied to practical determination of radiation exposures. These have been developed and will be published within two months as ICRU Report 51, Quantities and Units in Radiation Protection Dosimetry.

Also required for effective radiation protection are conversion factors for translation from calibration quantities to limiting quantities. Substantial progress has been made in developing the conversion factors, with major portions of the drafting work on the report on this subject now completed.

Vital to radiation protection activities is information on the fundamental interaction of radiation with body tissues, including interaction data for photons, electrons, protons and neutrons. This has now been provided via ICRU Report, 46, Photon, Electron, Proton and Neutron Interaction Data for Body Tissues.

To facilitate practical radiation protection activities, guidance is required on the implementation of measurement in terms of the defined operational quantities. This has been provided via ICRU Report 47, Measurement of Dose Equivalents from External Photon and Electron Radiations.

Phantoms constitute a key element in radiation protection measurements and work carried out via this project was aimed at specification of the vital properties of phantoms and the identification of phantoms that are appropriate for various uses. This work was brought to conclusion with the publication of ICRU Report 48, Phantoms and Computational Models in Therapy, Diagnosis and Protection.

In the case of radionuclides released to the environment, radiation protection for the public requires an effective method for the determination of the radionuclides deposited and *in situ* gamma spectrometry provides this. Work on this achieved major progress with the draft report on the subject receiving ICRU approval for publication as an ICRU report.

Protection against beta-ray exposure involves some special measurement problems. Major progress in the drafting work on the report on this subject was achieved during the current reporting period.

The assessment of the deposition of radionuclides in the human body also presents unique features of measurement. Important progress was achieved in the effort to develop a report on this subject with the formulation of a detailed outline for the report.

Radioactivity measurements are, of course, vital to many radiation protection activities. Work focused on the fundamentals of particle counting applied to radioactivity measurements is being carried out as part of this project. The preparation of the printer's manuscript for the resulting report is now being completed.

Among the physical data vital to radiation measurement is the stopping power for various radiation entities. Added to previously provided tabulations of stopping powers for electrons and photons is comparable information for protons and alpha particles, published as ICRU Report 49, Stopping Powers and Ranges for Protons and Alpha Particles.

An important source of public exposure to radiation, and hence a concern of radiation protection, is medical application of radiation. Thus, dose reduction in medical applications is important and a number of efforts are focused on this area. Work on each of these has made important progress with a report on performance assessment for various diagnostic modalities likely to enter the printing process soon. A report on prescribing, recording and reporting therapy doses is to be published within six weeks (ICRU Report 50).

Progress Report

Contract: FI3P-CT920001 Sector: A1B

Title: Collaboration on radiation dosimetry for radiation protection applications (EURADOS).

1) Dietze EURADOS

I. Summary of Project Global Objectives and Achievements

The European Radiation Dosimetry Group (EURADOS) was founded in 1981 to stimulate and improve cooperation of research in radiation dosimetry and related topics within Europe. The global objectives of EURADOS in the running project are:

1. The stimulation of collaboration in research and technical developments concerning the measurement and evaluation of exposures by ionizing radiation.
2. The harmonization of methods for assessing radiation exposures by means of intercomparisons, workshops and active collaboration between laboratories in Europe.
3. The collection and evaluation of physical data relevant to radiation dosimetry and to the assessment of occupational, accidental and environmental exposures.
4. The support of transfer of expertise and experience in radiation dosimetry from well experienced laboratories to other sites and young scientists in Europe by organizing seminars and tutorials.

About 180 scientists from about 65 laboratories participate in the work of EURADOS together with corresponding scientists from countries outside of the European Communities. Collaboration with scientist from eastern European countries becomes more important during the recent years and some scientist from these countries were invited to participate in Working Group meetings when financial support was available.

The work is mainly carried out by EURADOS Working Groups (WG), each of which consists of about 10 to 15 scientists from different European laboratories. Usually additional corresponding members and observers participate in the meetings of the Groups. The formation and scope of work is agreed by the EURADOS Council. Generally a Working Group will be set up to meet defined objectives and once these are achieved the Group will be disbanded. During the contract period 1992-1993 the following Working Groups have been active:

WG 2: Skin Dosimetry
WG 4: Numerical Dosimetry
WG 6: Assessment of Internal Dose
WG 7: Radiation Spectrometry at Working Environments
WG 8: Development of Individual Dosemeters for External Penetrating Radiation
WG 9: Criticality accident dosimetry
WG 10: Basic Physical Data and Characteristics of Gas Ionization Devices
WG 11: Radiation Exposure and Monitoring of Air Crews

Usually the Working Groups meet about once or twice a year in order to organize and coordinate their work, to discuss their results and to prepare recommendations etc. Often also subgroup meetings were performed. In order to increase the knowledge on and the cooperation between the Working Groups, in autumn 1992 all Working Group meetings were scheduled together on November 3-4 and performed at the same place in Paris. On November 5th a common meeting was used to present and discuss the further plans of the various Working Groups and to discuss the radiation protection research programme of the CEC as presented by H.G. Menzel.

The work of the groups will be described in detail in the following sections.

Workshops:

An international Workshop on "Individual Monitoring of Ionizing Radiation: The Impact of Recent ICRP and ICRU Publications" have been organized by EURADOS in cooperation with the PSI/Villigen, the DOE and the CEC at the Paul Scherrer - Institute/Villigen on May 5-7, 1993. The aim of the Workshop was to present and discuss the implications of the ICRP Publication 60 and the ICRU Report 47 for individual monitoring. The topics include basic questions on risk-related and operational radiation protection quantities, problems on properties of individual dosemeters, the quality assurance of individual monitors and practical problems of dosimetry services and record keeping. About 115 participants with 48 oral and poster presentations contributed to the success of the Workshop which gave also an important input to the work of the ICRP/ICRU Task Group for the revision of ICRP Publication 51.

Another Workshop on "Advances in Radiation Measurements - Applications and Research Needs in Health Physics and Dosimetry" Dose" is already in preparation and will be performed in October 1994 at Chalk River/Canada together with the AECL, Chalk River Laboratory.

Training Course:

EURADOS has started to organize training courses for young scientists in the fields of radiation physics, radiation dosimetry and radiation protection in order to increase their expertise and their knowledge of modern methods in radiation measurement and dosimetry.

A first Course ("Modern Methods in Radiation Measurement and Dosimetry") with 45 participants from 12 European countries have been performed in cooperation with the GSF/Neuherberg at Bad Honnef/Germany on November 23-27, 1992. During 5 days 22 lectures were presented by specialists of the various fields of detection and dosimetry of ionizing radiation ranging from radiation fields, basic quantities and principles of various types of dosemeter to new semiconductor devices and modern X-ray detectors at the ROSAT sattelite. Because of the extensive demand it is aimed to perform a more basic course together with the GSF at Neuherberg in September 1994 and another course similar to the first one in 1995.

A further Training Course on "Assessment of Internal Dose" has already been planned and will be performed in cooperation with CEA in Cadarache/France in spring 1994.

WG 2: Skin Dosimetry (Chairman: M. Charles)

The Working Group has concentrated their efforts on the following topics:

1. **Theoretical and experimental study of hot particle dosimetry**

   The measurement and dosimetry of hot particles is an important task and has been investigated in more detail during the current programme. Collaboration in theoretical and experimental studies has been initiated in order to estimate the absorbed dose in the skin in the vincinity of those particles. A Co-60 source, 200 μm in diameter, have been used and in addition to measurements with an extrapolation chamber radiochromic dye measurements were carried out in collaboration with Dr. Baum and Dr. Soares of the BNL and the NIST who are corresponding members of WG 2. The results were compared with calculations using either the semi-empirical VARSKIN code or a modified EGS4 Monte Carlo Code. The results of calculations were lower than the measurements by about a factor of 2 and there was only a poor agreement between the VARSKIN and the Monte Carlo code. Further measurements are in progress.

2. **ß-Dosimetry with extrapolation chambers**

   The dosimetry of low-energy ß-rays from a Pm-147 source has been extensively studied by intercomparison measurements with extrapolation chambers and further work is in progress using also a higher energy ß-source of Tl-204. The main efforts of the Working Group during the period considered concentrated on the production of a detailed report describing the measurement procedures, the results of the intercomparisons and the implications for ß-dosimetry protocols. The task will be finished and the planned report will be published in 1993/1994. The results became also important for the forthcoming ICRU Report on "Dosimetry on external beta rays for radiation protection", on which many members of the Working Group contributed very actively.

3. **Benchmark experiment on the determination of dose rates from a Sr-90/Y-90 source**

   The main purpose of this programme was to adapt a Monte Carlo code for beta dosimetry. A Sr-90/Y-90 beta source with accurately known properties was used for measurements and its data were also used in the simulation by a computer programme of Patau. While measurements were performed at PTB (Böhm) and CEA (Herbaut), Patau calculated the dose rates for the same geometries with a code developed to run on a PC. Final results will soon been available and they will be published in 1994.

4. **Collaboration with EULEP**

   Collaboration with EULEP on studies of biological effectiveness of different radiations and depth of sensitive layers in the skin has been continued during the period considered. In accidental dosimetry with very high doses of low-penetrating radiation the doses at depths of 2 to 3 mm becomes important. Investigations during the report period have been concentrated on Co-60 and its effect on pig skin. Particles of 200 μm diameter or less with an activity of about 20 μCi have been produced in order to study whether the ICRP dose limit for 'hot particles' (average dose of 0.5 Sv over 1 cm^2 at 70 μm depth) is appropriate in the case of Co-60 radiation. The investigations have not been finished yet.

WG 4: Numerical Dosimetry (Chairman: B.R.L. Siebert)

During recent years computational work becomes more and more important in radiation dosimetry. Measurements of doses in phantoms are often very difficult and nearly impossible in human bodies. On the other hand dose distributions and conversion coefficients are fundamental data necessary for the definition of risk-related equivalent dose quantities and operational dose equivalent quantities. For this reason the Working Group became increasingly important during the last year in supporting the work of the ICRP/ICRU Task Group for the revision of ICRP Publication 51 as a consequence of ICRP Publication 60.

In detail, the Working Group deal with the following topics during the report period:

1. **Support of the ICRP/ICRU Task Group**

   As the consequence of ICRP Publication 60 many conversion coefficients published in ICRP 51 must be revised. The Working Group supported this work strongly by encouraging various people to participate in this urgent task and by coordinating the calculation of new data for neutrons, photons and electrons.

   Due to the new weighting factors and quality factors all neutron data have to calculated again, especially organ equivalent and effective doses. While various members of the group contributed to the calculation of neutron data, Dietze and Siebert (PTB) collected all these data in order to obtain an evaluated set of mean data as a basis for the ICRP report. It has been agreed that the full evaluation procedure with all details will be published by the EURADOS Working Group in 1994.

   Most of the photon data had already been calculated by the GSF group. Hence much less coordination was necessary for the photon data compared to neutrons.

   New calculations had also to be carried out for electron radiation. Chartier (CEA) coordinated this work, while other members performed many calculations. Results will directly go into the data set for the planned ICRP Publication.

2. **Questionaire on computer methods used in dosimetry**

   The aim of this activity is to collect and distribute information on the use of computer programs for dosimetric problems and to initiate bench mark tests and intercomparison calculations with different codes in order to obtain some quality assurance. This was aimed to be achieved firstly by the evaluation of a questionnaire which was sent to laboratories in Europe in 1991. After having recieved 42 replies in 1992 a careful study has resulted in the decision of the Working Group to prepare a revised and extended questionaiere which should be distributed in all countries of the CEC by Working Group members and in addition published in Radiation Protection Dosimetry. The questionaire will be analysed by a subgroup shared by M. Terrisol (Toulouse) and the results will be published in 1994.

3. Multisphere response function calculations

The calculations for this work have now been completed and a report for publication in Radiation Protection Dosimetry is in progress. The MCNP code can well be used for the calculation of response functions for detectors based on moderating devices for neutron energies from thermal up to 20 MeV. An extension of the energy range to higher neutron energies is, however, very desirable.

4. Electron transport codes

For the project period it is aimed to collaborate in the study and intercomparison of electron transport codes. Such codes are becoming more generally available and because of the increasing power of personal computers they are more widely used by non-specialists who are not aware of the approximations included in those codes. The Working Group will study this situation, but because of the urgent tasks for the conversion coefficients this work is delayed and has only just been started.

Publications:

1. C.A. Perks, D.J. Thomas, B.R.L. Siebert, S. Jetzke, G. Hehn and H. Schraube. Comparison of Response Function Calculations for Multispheres. Radiat. Prot. Dosim. 44 (1992) 85
2. B.R.L. Siebert, E. Dietz, S. Jetzke. Comparison of Measured and Calculated Bonner Spheres Responses for 24 and 144 keV Incident Neutron Energies. Radiat. Prot. Dosim 44 (1992) 89
3. B.R.L. Siebert, H. Schraube, D.J. Thomas. Catalogue of Neutron Fields in Radiation Protection Environments and Programmes to obtain Appropriate Calibration Factors for Neutron Dosimeters. Radiat. Prot. Dosim. 44 (1992) 150
4. B.R.L. Siebert. Radiation Quantities: Their Interrelationship. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen May 5-7, 1993 (to be published in Radiat. Prot. Dosim.)
5. M. Marshall, D.J. Thomas, C.A. Perks, O.F. Naismith. Radiation Quantities: Significants of the angular and energy distribution of the radiation field. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen May 5-7, 1993 (to be published in Radiat. Prot. Dosim.)
6. G.F. Gualdrini, F. Padoani. Dose Equivalents per Unit Fluence for Tissue Equivalent Slab Phantoms for Electrons from 50 keV to 10 MeV. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen May 5-7, 1993 (to be published in Radiat. Prot. Dosim.)

WG 6: Assessment of Internal Dose (Chairman: T. Gibson)

The determination of dose after an intake of radioactive material is an important task in radiation dosimetry. To continue to improve collaboration in internal dosimetry within Europe, the Working Group members contribute actively to the following projects in the period 1992-1993.

1. **Stable Isotope studies**

   The Working Group participates in the work on stable isotopes for use in metabolic studies. While strontium and tellurium have been chosen for first studies the work has now being extended to other stable elements e.g. Co. Work has been carried out at Harwell to find analogues for the transuranics for which stable isotopes are not available. They had investigated the lanthanide series elements. Little difference between mambers of the series had been found but observed uptake values were again greater than quoted by ICRP ($>10^{-3}$ rather than 10^{-4}).

2. **Intercomparisons**

   Studies of dose assessments following the intake of radioactive material have been continued in order to reduce the variation between results from different laboratories in Europe obtained during former intercomparisons. The results of the first intercomparison have already been published. A second intercomparison is planned. It is aimed to use examples of Tc-99 and tritium intakes or potentially plutonium inhalation.

   A further initiative has been started to perform an intercomparison for whole-body monitors using a small phantom equivalent to a 4 yr old child.

3. **European Registries**

   The Working Group has further supported the collaboration in setting up European Registries for Internal Dosimetry which include assessment data, autopsy data and models. While in the U.K. the national project is in progress, an extension to Europe remains difficult. An IAEA Technical Committee had also been set up to assess the feasibility of establishing such registry. No strong progress have been achieved by the WG during the report period.

4. **Joint EULEP/EURADOS Task Group on the Human Lung**

   The cooperation with EULEP in the Human Lung Task Group has been continued. The implications of the proposed ICRP lung model for dose assessments is investigated and the sensitivity of predicted doses to variations in model parameters is assessed. At the fourth Task Group Meeting on October 5/6, 1992 it has been reported in detail about the activities of the Group (see ref. 1)

Publications

1. Bailey, M.R. ICRP Respiratory Tract Dosimetric Model: Deposition and Clearance. EULEP Newsletters 73 (1993) 15

1. Gibson, J.A.B, Birchall, A., Bull, R.K., Henrichs, K., Iranzo, C.E, Lord, D.J.,Piechowski, J., Sollett, E., Tancock, N.P. and Wernli, C. European Intercomparison of Methods Used for the Assessment of Systemic Burdens of Internally Deposited Radionuclide. Radiat. Prot. Dosim. 40 (1992) 245

WG 7: Radiation Spectrometry at Working Environments

(Chairman: H. Klein)

In neutron or mixed neutron-photon dosimetry the lack of any detailed knowledge of the neutron spectrum in the majority of workplaces introduces major uncertainties into both area and personal dosemeter readings. Further uncertainties are introduced when estimating individual dosimetric quantities, for both neutrons and photons, by lack of knowledge of the directional dependence of the radiation incident on a human body. Both problems are under consideration by this Working Group. In 1992 the work were concentrated on the study of the properties of the various types of spectrometers which were available to the various participants of the WG.

Six members of the WG have access to a Bonner Sphere spectrometer system. General progress have been achieved in the calculation of response functions. The MCNP transport code appears particularly suitable for this purpose and measurements have verified the recent calculations. Progress have also been made in the development of better unfolding codes, however, the unfolding system remains underdetermined, because of the limited number of Bonner spheres available.

Five members of the WG use also recoil proton counters for the analysis of neutron energies in the range from 50 keV to 2 MeV.

In addition four members used NE-213 liquid scintillation detectors for neutron spectrometry above 500 keV neutron energy.

Other spectrometric systems available were NaI-, BGO- and Ge-Detectors for photon spectrometry and also microdosimetric detectors for mixed-field measurements.

The main task of the WG is the investigation and intercomparision of the various spectrometric systems in realistic neutron or mixed neutron-photon fields. During the report period intercomparison measurements have been organized and performed by the WG at three different sites.

1. CEA Facility at Cadarache

The available neutron field at the Cadarache facility includes 14-MeV and fission neutrons moderated by a sphere of iron and polyethylene. MCNP calculations have been performed for modelling the mixed neutron-photon field at the reference position. Measurements have already been performed by three teams (CEA, GSF and PTB) using a range of spectrometers, two TEPCs, several area survey instruments and both albedo and etched-track personal dosemeters. The results of the various spectrometers agree fairly well, although problems became evident when using calculated response functions from literature for a LiI-based Bonner Sphere system. While the TEPCs and the personal dosemeters tended to under-read by a few tens of percent on average, the area monitor results show relative deviations from -10% up to 50 %.

2. Measurements at Ringhals and Oskarsham/Sweden

In December 1992 intercomparison measurements were performed at two different sites in Sweden, a nuclear power reactor at Ringhals and at a nuclear fuel storage area at Oskarsham. The project was performed in strong cooperation with the Swedish Radiological Protection Institute which partly sponsored the measurements. Eight groups participated in the intercomparison measurements which became a very stringent test for the spectrometer systems, since the conditions involve very high dose rates, unusual environmental conditions and also strict time limitations at the reactor site. While the results have not been evaluated to date, it became obvious that the strongest differences in the results of the different systems were unexpectedly obtained for the photon dose component of the mixed radiation field. Further studies are necessary to solve this problem.

3. Measurements at AEA Technology, Winfrith, U.K.

From February 22, to March 12, 1993 a measurement campaign have been performed at the ASPIS test facility on the NESTOR reactor at Winfrith, U.K. At the test facility a variety of different spectra based on a moderated fission source can be produced and the dose rate can be varied over a wide range. A further advantage of this facility is that the spectra can be calculated using validated neutron transport codes.

In the first campaign a hard fission spectrum was realized at the test site which simulates the spectrum at the containment of a PWR. Low energy albedo neutrons were additionally realized by a thick polyethylene slab as backscatter. The measurements with various spectrometer systems were performed in strong cooperation with the Winfrith Technology Center (Mr. Murphy). The evaluation of the results are still in progress.

Publications

1. M. Marshall, D.J. Thomas, C.A. Perks, O.F. Naismith. Radiation Quantities: Significants of the angular and energy distribution of the radiation field. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen May 5-7, 1993 (to be published in Radiat. Prot. Dosim.)

Further publications describing the various intercomparison measurements and discussing the results and consequences of thes measurements are in progress. Papers will be published in 1994.

WG 8: Development of Individual Dosemeters for External Penetrating Radiation (Chairman: J. Harvey)

Individual monitoring in mixed neutron/photon fields becomes an increasing problem, when the new ICRP recommendations are introduced into practice, because the sensitivities of existing individual dosemeters are mostly not sufficient. This and many other problems regarding individual monitoring and the application of personal dosemeters have been discussed recently at the Workshop on "Individual Monitoring of Ionizing Radiation: The Impact of Recent ICRP and ICRU Publications" at Villigen (Switzerland) on May 5-7, 1993. The Workshop has been organized by the Working Group 8 of EURADOS in cooperation with the CEC, the DOE and the Paul Scherrer Institute/Villigen.

During the reporting period the efforts of the WG were strongly concentrated on the preparation and the realization of this Workshop. Many members of the WG participated actively with oral or poster contributions in this Meeting.

Furtheron the Working Group deals with the following tasks:

1. Various groups in Europe are using track-etch detectors in neutron dose measurements. In this context they have developed many different procedures in the evaluation of irradiated polycarbonate foils. A detailed intercomparison has been performed and the final evaluation of the data is still in progress.
2. With respect to sensitivity the development of new detectors, like e.g. bubble detectors, looks promising. An analysis of the advantages and disadvantages of new detector designs is in progress and it is aimed to include newly available detectors in future intercomparisons.
3. The calibration procedures in individual monitoring are still under discussion. The Working Group contributes to the discussion of the methods proposed by ICRU and will make recommendations to improve harmonisation within the European Communities.

Publications:

1. Harvey, J.R. A three Element track-edge Neutron Dosemeter with good Angular and Energy Response. Radiat. Prot. Dosim. 44 (1992) 325
2. Alberts, W.G. International Study of CR-39 Track-Etch Neutron Dosemeters. Radiat. Prot. Dosim. 44 (1992) 323
3. Alberts, W.G. Investigation of Neutron Individual Monitors on the Basis of Etched-track Detectors. The 1990 EURADOS-CENDOS Exercise. PTB-Report PTB-N-10 (1992)
4. T.O.Marshall, C.O. Wernli. Performance Requirements of Personal Dosemeters: Can these met by Present or Future Designs? Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen May 5-7, 1993 (to be published in Radiat. Prot. Dosim)
5. D.T.Bartlett, W.G. Alberts. Type Testing and Calibration of Personal Dosemeters. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen, May 5-7, 1993 (to be published in Radiat. Prot. Dosim)
6. J.R. Harvey, G. Portal. Quantities in Radiation Protection, Their Functions and Roles. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen, May 5-7, 1993 (to be published in Radiat. Prot. Dosim)
7. M. Marshall, D.J. Thomas, C.A. Perks, O.F. Naismith. Radiation Quantities: Significance of the angular and energy distribution of the radiation field. Presented at the Workshop on Individual Monitoring of Ionizing Radiation, Villigen, May 5-7, 1993 (to be published in Radiat. Prot. Dosim)

WG 9: Criticality accident dosimetry (Chairman: R. Medioni)

The working group coordinates investigations of the quality of criticality accident dosimetry. This work concentrates on the preparation and performance of intercomparison measurements at the SILENE reactor at Valduc/France. In the preceding period 1990-1992 the efforts had been concentrated on the investigation of the radiation field properties at the reference positions at a distance of 4 m from the reactor core, where the intercomparison measurements should be carried out. The results of the spectrometric and dosimetric measurements showed that the neutron and gamma-ray field at the arc in 4 m distance was generally uniform within ±5% and therefore sufficient homogeneous for the intercomparison. Independent dose measurements are in good agreement and will allow to specify a reference value for the intercomparison.

From 7th to 18th June, 1993 the intercomparison measurements on criticality accident dosimetry systems took place at the SILENE reactor. Laboratories from 14 countries participated in the measurement programme. This action was additionally sponsored by the CEC, DG XI, the IAEA and DOE.

Two different radiation fields were realized. Irradiations were performed with radiation from the bare reactor core and from the core surrounded by a lead shield. The intercomparison includes exposure of dosemeters free in air and in front of anthropomorphic phantoms. The evaluation of the results is still going on. It is aimed to prepare a comprehensive report which will include the reference dosimetry, the spectrometry measurements, brief descriptions of the criticality accident dosimetry systems used by the participants, the measured dose data and an overall analysis of the results. The report will be published in 1994.

On the basis of this report and the results of the intercomparison it is aimed to perform a workshop on accident dosimetry in cooperation with the CEC, CEA und IAEA, where results and implications will be discussed.

Publications:

It has been agreed by the participants of the intercomparison not to publish any detailed data of the radiation field used in the intercomparison before the participants have delivered their final results of the intercomparison measurements. Hence no direct publication of the work can be presented until now. Reports are still in progress.

WG 10: Basic Physical Data and Characteristics of Gas Ionization Devices

(Chairman: P. Pihet)

The general objective of the group is the assessment of basic physical data relevant to the biological effect of ionising radiation and the development of instrumentation for dosimetry in radiation protection and radiobiology, with special emphasis on gas ionisation devices, e.g. proportional counters (PC) and ionization chambers. The progress achieved during the report period are concerned with the following topics:

1. **Basic data relevant to the measurment of ionising radiation**

   W-values for neutrons:
   A large progress was achieved for the evaluation of W-values for protons. Using most recent W-data from 1 keV to 5 MeV a new analytical representation for W(E) in methane based TE gas was investigated. Different expressions were discussed by applying a variance analysis to the data sets. The results of Siebert et al. shows that the new fit allow W for protons to be represented with a relative standard deviation of 2,5 % and is proposed to replace the older ones. It shows a distinct bump at around 200 keV which, however, critically depend an the asymptotic W-value. The new fit has a significant influence on the interpretation of the energy deposition as measured with a PC.

   Electron molecule cross sections:
   The central motivation of the task is to provide reliable electron collision cross sections in organic gases focussed on the properties and the application of proportional counters. A substantial progress was achieved at Toulouse University to determine cross section data in methane by combining experimental swarm parameters analysis and electron discharge modelling. The work was continued by determining electron molecule cross sections in different gases, namely isobutane and by calculating gas gain characteristics of proportional counters filled with gas mixtures. In a second step experiments are performed at Legnaro INFN Laboratory to verify the gas gain modelling calculations by measurements with a cylindrical proportional counter using a low energy ion beam for scanning.

2. **Characteristics of low pressure proportional counters**

   Although low pressure proportional counters (PC) are widely applied in dosimetry and microdosimetry, the knowledge of their basic characteristics remained for long time rather poor due to the lack of adequate understanding of the charge collection processes involved. Progress has now been achieved in simulating the gas gain processes and in performing microscopc calculations for low pressures using Monte Carlo techniques.
   Dose equivalent responses of TEPCs operated at simulated diameters below 1 μm was investigated for neutrons from 10 keV to 1 MeV (Universität Homburg/Saar).
   Other studies were performed in order to increase the sensitivity of TEPCs either by enlarging the counter size (AECL) or by using a multi-cellular geometry (IPSN).

3. **Design, construction and use of low pressure proportional counters**

   The project to edit a comprehensive report on the design and the construction of low pressure proportional counters originated one year ago has been continued. The work is performed in close collaboration with colleges from the US (P. Kliauga, L. Braby, G.W. Johnson). Editorial tasks were organized and first drafts of some chapters have been already discussed on a WG 10 meeting at the KFA Jülich on May 16-19, 1993.

4. **Intercomparison of TEPC systems in real radiation fields**

The Working Group participated with 5 TEPC systems in the intercomparison measurements organized by WG 7 at the nuclear power reactor site at Ringhals and a nuclear fuel storage area at Oskarsham in Sweden. The evaluation of the results is in progress.

5. **Organization of a Workshop**

The Working Group has organized to perform a Workshop on "Advances in Radiation Measurements - Applications and Research Needs in Health Physics and Dosimetry" at Chalk River , Ontario, Canada on October 1994. This Workshop will be held in strong collaboration with AECL Research in Canada and the CEC, DG XII. The Workshop will deal with gas ionization devices and other innovative instruments for assessing and monitoring radiation doses. It will especially focus on active devices and on measurement techniques. A first announcement has been prepared and distributed.

Publications

1. Boutrouche, B., Bordy, J.M., Barthe, J., Segurr, P. and Portal,G. New concept of a minicounter for individual neutron dosimetry. Radiat. Prot. Dosim. 45 (1992)
2. Collauti, P., Conte, V., Talpo, G, Tornielli, G. and Bouchefer, M. A method to investigate the working charackeristics of new microdosimetric detectors which use low gas pressures. Nucl. Instr. Meth, A321 (1992) 392-402
3. Collauti, P., Talpo, G, Tornielli, G., Ackerman, A. and Breskin, A. Measurement of ionisation distribution around a particle track at a nanometer level. Radiat. Prot. Dosim. 45 (1992)
4. Pihet, P., Gerdung, S., Grillmaier, R.E., Kunz, A. and Menzel, H.G. Critical assessment of calibration techniques for low pressure proportional counters used in radiation dosimetry. Radiat. Prot. Dosim. 44 (1992) 115-120
5. Pihet, P. Données physiques de base et amélioration des detecteurs à gaz pour la mesure de l'ionisation et de l'energie absorbée dans la matière. Radioprotection 27 (1992) 320-323
6. Schrewe, U.J., Brede, H.J., Gerdung, S., Kunz, A., Meulders, J.P., Nolte, R., Pihet, P. and Schuhmacher, H. Determination of kerma factors of A-150 plastic and carbon at neutron energies between 45 and 66 MeV. Radiat. Prot. Dosim. 44 (1992) 21-24
7. Schuhmacher, H., Brede, H.J., Henneck, R., Kunz, A., Meulders, J.P., Pihet, P. and Schrewe, U. Measurement of neutron kerma factors for carbon and A-150 plastic at neutron energies of 26.3 and 37.8 MeV. Phys. Med. Biol. 37 (1992) 1265-1281
8. Schuhmacher, H. Tissue equivalent proportional counters in radiation protection dosimetry: expectations and present state. Radiat. Prot. Dosim. 44 (1992) 199-206
9. Siebert, B., Grindborg, J.E., Großwendt, B. and Schuhmacher, H. New analytical representation of W values for protons in methane based tissue-equivalent gas. Radiat. Prot. Dosim. 45 (1992)
10. Segur, P., Collautti, P., Moutarde, C., Conte, V., Talpo, G, and Tornielli, G. Comparison between experimental and theoretical determination of the space variation of the gas gain inside low pressure TEPCs. Prot. Dosim. 45 (1992)

WG 11: Radiation Exposure and Monitoring of Air Crews

(Chairman: I.R. McAuly)

On November 1992 the EURADOS Council has decided to set up a new Working Group which will be concerned with the cosmic radiation to which air crews are exposed and the scientific and technical problems associated with the dosimetry on aircrafts and air crew monitoring. The group works in strong cooperation with DG XI and DG XII of the CEC. The main reasons for setting up this WG are:

1. The 1990 recomendations of the International Commission on Radiological Protection (ICRP Publication 60) includes the exposure in jet air crafts to natural radiation sources as part of occupational exposure.
2. New and more restrictive limits on the effective dose for occupational exposure of workers are recommended.
3. The radiation field in the higher atmosphere differs strongly from usual environmental radiation fields at ground and radiation monitoring is therefore more difficult and needs specific consideration.
4. The scientific community, which has been ask for information by professional organizations and airlines, could not always supply consistent answers. DG 11 expects to obtain clear recommendations from the WG concerning the dosimetry in air crafts and the monitoring of air crews.

At a first meeting in Luxembourg on March 22-23, 1993 the detailed tasks of the WG have been discussed. It is aimed to prepare a report within two years which should contain information on dose eqivalent values at flight heights, variations of the radiation field and a clear recommendation how to perform the radiation monitoring in aircrafts and for air crews.

Three subgroups were installed in order to prepare drafts for parts of the planned report. They deal with the following topics:

1. **Assessment and evaluation of previous works.**
   Many investigations have already been performed on the radiation field in the higher atmosphere and it is necessary to carefully evaluate these data in order to obtain a sound basis for recommendations.
2. **Dose quantities.**
   Because of the unusual properties of the radiation field in the higher atmosphere it must be carefully checked, if the operational radiation protection quantities proposed for use at ground are applicable for the radiation monitoring of air crews. Alternative possibilities must be studied and discussed.
2. **Standard fields, reference instruments, calibrations.**
   Radiation monitoring in aircraft needs instruments which must be calibrated in fields appropriated to those where these instruments will be used. The choice of reference instruments must be discussed and a recommendation should be given how instruments have to be calibrated.

For the meeting in January 1994 the subgroups will prepare first drafts.

Progress Report

Contract: **F13P-CT920040** **Sector: A11**

Title: Accident dosimetry in populated areas: the use of solid-state dosimetry techniques with ceramics and other natural materials.

1)	Bailiff	Univ. Durham
2)	Goeksu	GSF
3)	Stoneham	Univ. Oxford
4)	Bøtter-Jensen	Lab. Risø

1. Summary of Project Global Objectives and Achievements

Project Objectives

1. To identify ceramic and other natural materials (such as teeth) which are suitable for retrospective accident dosimetry using solid-state techniques.
2. To ascertain appropriate experimental procedures for dose evaluation.
3. To evaluate transient fall-out dose in the sampled study areas down-wind of Chernobyl fall-out in the Ukraine and southern Belarus. To derive shielding factors by comparing dose determinations for interior and exterior samples.
4. To identify factors which affect minimum resolvable fall-out dose.
5. Integration of the results obtained in 1) - 4) above to evaluate the capability of solid-state dosimetry techniques for retrospective accident dosimetry in populated areas

Achievements at Midterm

- The group has achieved good overall progress in the Stage I of the project which is concerned with methodological aspects of the application of luminescence and EPR techniques. This represents a consolidation of previous work, now published, which demonstrated the feasibility of dose evaluations with a selection of materials from the Exclusion Zone.
- Preliminary work on an investigation of dose as a function of depth in brick has been completed and this will be further developed in Stage II. Given the transient nature of the incident radiation dose and the potential for large variations in energy spectra of charged particles and photons, the choice of sample location represents an important part of the study.
- Significant improvements in EPR detection levels have been achieved by removing the organic fraction of bone and the procedure developed will be applied to teeth to investigate extension of the technique to dose levels below 0.3 Gy. Of ten other types of sample tested with EPR, five were found to be of potential interest for dose determinations below 1Gy comprising: cane sugar, blackboard chalk, boiler scale, egg shells and baking powder.
- New luminescence techniques using visible and infra-red stimulation have been investigated. The preliminary results are very encouraging, indicating higher detection sensitivity and improved precision. Both fundamental and empirical aspects of their application are being studied.
- An interlaboratory source calibration has been completed. Sized fractions of quartz were irradiated at the secondary standards laboratory with a ^{60}Co photon source and distributed to each laboratory for dose evaluation.
- The suitability of a number of non-ceramic domestic materials were evaluated using TL and EPR. Of six types tested with TL, blackboard chalk, boiler scale and dust were found to be the most suitable with lower dose limits in the region of 0.3-0.5 Gy.
- A field trip has been completed during July/August which enabled locations within the Exclusion Zone in Belarus and the Ukraine to be visited and their potential for dosimetry sampling assessed. Valuable new contacts with scientists working in institutes in Minsk and Kiev and existing collaborative links with Chernobyl were consolidated.

Head of Project 1: I.K. Bailiff

II:. Objectives for the reporting period (Stage 1)

i) Review of experimental procedures for luminescence measurements.
ii) Measurement of luminescence characteristics of ceramic samples for dosimetry measurements.
iii) Inter-laboratory calibration of laboratory radiation sources.
iv) Experimental designs for dose-depth measurements in ceramic samples.
v) Measurement of optically stimulated luminescence at room and low temperatures using a tunable laser source.
vi) Complete fieldwork for the assessment of sampling locations and exploration of future collaboration with scientific groups in the study area of Ukraine and Belarus.

Objectives for Stages 2 & 3:

i) Inter-laboratory comparison: determination of dose-depth profiles in ceramic using secondary standard sources and beams of differing particle and photon energies, with particular attention to the effect of low energy photons.
ii) Characterization of luminescence and properties of field samples using thermal and optical stimulation, including spectral emission.
iii) Interlaboratory comparison: measurement of accrued dose in field samples.
iv) Consolidation of fieldwork and laboratory documentation archive.
v) Preparation of publications and Final Report

III. Progress achieved including publications

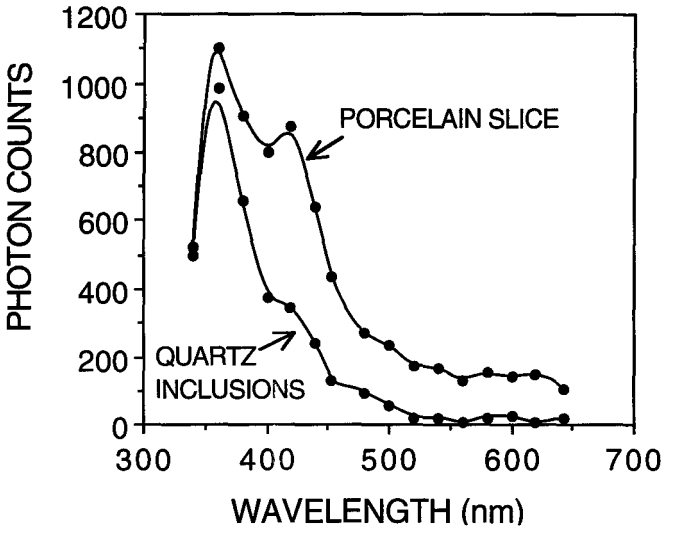
Dr S. Petrov was appointed to the project towards the end of stage 1 and thus has only recently been able to start making his contribution to the project. His expertise in solid state physics applied to radiation detectors is expected to provide valuable assistance to the project. He will also be able to help accelerate communications with scientists in the FSU.

Thermoluminescence properties of ceramics

The measurement of spectral emission is often neglected in thermoluminescence measurements because of the requirement of specialised spectrometers with high sensitivity. They are important for a number of reasons including: changes in the emission spectrum will lead to erroneous dose evaluations if they are not accommodated within the detection window; valuable sensitivity may be lost by using an inappropriate spectral window for detection; they give specific information concerning radiative recombination and consequently are a sensitive indicator of the components of emission.

The pre-dose technique, which is the most sensitive of the TL techniques for dose evaluation using ceramics, is based on the sensitization of the 100 °C TL peak. TL emission spectra have been measured for samples of porcelain and quartz grains extracted from floor tile - both from Pripyat. Examples are shown in the figure below showing differences between the two materials which are being investigated further.

An invited paper reviewing the use of the pre-dose technique was given at a recent conference.



Optically stimulated techniques

The development of a laser facility during 1993 based on the use of a Ti:sapphire solid state laser pumped by an Argon-ion gas laser has provided a powerful tunable IR source for investigation of optically stimulated luminescence in minerals using highly monochromatic radiation. The properties of feldspars have been investigated at temperatures ranging from 77K to 300K using a continuous flow cryostat. The results are being used to form the basis of our understanding of the details of charge storage, charge release and luminescence recombination which are of importance in developing the use of OSL for dosimetry. Infra-red stimulated luminescence has been studied in feldspars; the laser output is sufficiently high (1W) to investigate its use with other minerals .

Measurement of the luminescence decay curve under optical stimulation has shown that the form of decay is not first order. The effect of the kinetics of decay on the use of various measurement procedures for dose evaluation, such as a single aliquot procedure, is being studied.

Inter-laboratory calibration

Samples of quartz, which had received a dose of 3Gy at the GSF Secondary Standards Laboratory from a ^{60}Co photon source, were measured in the laboratory's TL reader. Using the high temperature signal, the mean absorbed dose was evaluated using previously calibrated $^{90}Sr/^{90}Y$ irradiators and found to be within 2% of the known GSF dose. This was reassuring given our current estimate of source calibration accuracy of ±6%.
A detailed report has been prepared for the Project.

Fieldtrip

A visit to Chernobyl (Pripyat), Kiev (Institute of Radiation Medicine) and Minsk (Institute of Radiobiology) took place between July 22nd and August 5th; the Project's PECO Action

partner laboratory (Estonian Academy Sciences) played an important role in making local arrangements. The visit proved to be very successful and agreement in principle was reached regarding collaboration in the area of retrospective dosimetry. Evacuated dwellings in the Exclusion Zone within Belarus and the Ukraine were examined for suitable samples and the areas generally surveyed for other suitable dosimetric materials. Power line insulators are being considered as a new candidate for testing. The apartment buildings in Pripyat of interest to the modelling group at GSF were also examined for potential samples with a view to future comparative work.

Automated measurements
Discussions with a potential sub-contractor regarding the devlopment of a new experimental control and data analysis system which will be specifically tailored for the type of semi automated TL reader used in each of the partner laboratories. This work is aimed at giving each laboratory the capability to perform the complex experimental routines and specialised data analysis required for dose evaluation with modern ceramic samples.

Additional coordination functions
The Estonian Academy of Sciences was successful in obtaining support under the EC's PECO Action and the Luminescence Dosimetry Laboratory will be contributing to both measurement studies and in arranging fieldwork. The administrative difficulties in arranging contracts with the relevat PECO Action offices have been formidable, requiring a disproportionate amount of project time.

After discussions with the modelling group at GSF led by Dr P. Jacob, an application was submitted to join ECP10 under the *Health consequnces of the Chernobyl Accident* programme. The project aims to develop procedures for comparing dose estimates obtained with solid-state dosimetry and computer-aided modelling calculations.

Publications relevant to project:

Bailiff, I.K (1992) Measurement of the stimulation spectrum (1.2-1.7 eV) for a specimen of potassium feldspar using a tunable solid state laser. Radiat. Prot. Dosim. **47**, 307-311.

Hütt G., Brodski L., Bailiff I.K., Göksu Y., Haskell E., Jungner H. and Stoneham D. (1993) Accident dosimetry using environmental materials collected from regions downwind of Chernobyl: A preliminary evaluation. Radiat. Prot. Dosim. **47**, 307-311.

Stoneham D., Bailiff I.K., Brodski L., Göksu H.Y., Haskell E., Hütt G., Jungner H. and Nagatomo T. (1993) TL Accident dosimetry measurements on samples from the town of Pripyat. Nucl. Tracks Radiat. Meas. **21**, 195-200.

Bailiff, I.K. The pre-dose technique. Submitted for publication in Nuclear Tracks and Radiation Measurements (August 1993).

Bailiff, I.K, and Barnett, S.M. Characteristics of infra-red stimulated luminescence from feldspars at low temperatures. Submitted for publication in Nuclear Tracks and Radiation Measurements (August 1993)

Haskell, E.H., Bailiff, I.K., Kaipa, P.L. and Wrenn, M.E. Thermoluminescence measurements of gamma-ray doses attributable to fallout from the nevada test Site using building bricks as natural dosemeters. Submitted for publication in Health Physics (August 1993).

Head of project 2: Dr. H.Y. Göksu

II:. Objectives for the reporting period

The project is conducted with three main components for this reporting period.

1. Investigation of thermoluminescence (TL) properties of various house hold natural products to select suitable material for retrospective dose assessment in application to accident dosimetry.

2. Investigation of the applicability of the EPR method for various household material as well as the evaluation of the applicability of EPR method for individual dose assessment.

3. Organisation and preparation of suitable material for inter-laboratory source calibration for all the participating laboratories.

III. Progress achieved including publications

The materials used at this stage of the investigation were similar or commercially available products collected from Pripyat.

1. Radiation induced thermoluminescence response of black-board chalk ,boiler-scale , egg shell , dust in closed cupboards, the table salt and sodium bicarbonate are investigated in terms of their lowest limit for dosimetry. It is found that black board chalk and boiler scale can be used to detect doses above 0.30 Gy and dust collected from closed cupboards can be used above 0.50 Gy. The other material are found not to be applicable. The preliminary results are presented in Table 1.

2. The radiation induced EPR signal in various material are investigated. The samples presented in Table 2 are found not to be suitable for retrospective dosimetry. Table 3 list the results of the preliminary investigations on material which have potential applicability at doses below 1 Gy.

Improvements were made in-vitro dosimetry by removing the organic component in bone. Similar treatment will be applied to teeth to investigate the applicability of teeth for individual dose assessment below the 0.30 Gy.

3. Fine granular quartz is prepared and investigated for the suitability for inter laboratory calibration. After necessary stabilisation procedures, the samples are irradiated at secondary standard laboratory at GSF and distributed to all participating laboratories who are using thermoluminescence and/or optically simulated luminescence techniques on ceramics. The evaluation of the results will be presented in the next reporting period.

Publication

Göksu H.Y., Regulla D. and Vogenauer A. (1993) Reconstruction of gamma dose distribution in salt at radioactive waste disposal site by the water insoluble fraction. Radiat..Prot. Dosim..**47,** 331-333.

Hütt G., Brodski L., Bailiff I.K., Göksu Y., Haskell E., Jungner H. and Stoneham D. (1993) Accident dosimetry using environmental materials collected from regions downwind of Chernobyl: A preliminary evaluation. Radiat. Prot. Dosim. **47**, 307-311.

Stoneham D., Bailiff I.K., Brodski L., Göksu H.Y., Haskell E., Hütt G., Jungner H. and Nagatomo T. (1993) TL Accident dosimetry measurements on samples from the town of Pripyat. Nucl. Tracks Radiat. Meas. **21**, 195-200.

Wieser A., Haskell E., Kenner G. and Bruenger F. (1993) EPR dosimetry of bone gains accuracy by isolation of calcified tissue. Appl. Radiat. Isot. (in print).

Wieser A., Göksu H.Y., Regulla D. and Vogenauer A..(1993) Limits of retrospective accident dosimetry by EPR and TL with natural materials. 7th International Specialist Seminar on TL and ESR Dating. 5-9 July 1993 Krems, Austria. .

Table 1: Useful materials for retrospective radiation accident TL dosimetry.

sample	TL-peak, ℃	lower dose limit, Gy	remarks
black-board-chalk	300	0.3	depends on the country of origin
boiler-scale	280	0.3	independent on origin
dust in closed cupboards	180	0.5	organic dust should be eliminated
table salt (un-dissolved fraction)	220	0.5	applicable with certain Trade Marks

Table 2: Useful materials for retrospective radiation accident EPR dosimetry.

sample	EPR signal, g-value	lower dose limit, Gy	thermal stability	UV-stability
cane-sugar	2.0048	0.5	150°C, $T_{1/2}$=3h	change in spectrum after 3 h
boiler-scale	2.0005	0.5*	150°, $T_{1/2}$=5h	$T_{1/2}$=2h
egg-shells	2.0018	0.5	150°C, $T_{1/2}$=12h	no influence
black-board-chalk	2.0085	2*	150°C $T_{1/2}$=3h	$T_{1/2}$=2d
baking-powder	2.0075 and 2.0025	3	100°C, T1/2=4h	no influence

* dependent on origin of the sample.

Table 3: Materials found not useful for retrospective radiation accident EPR dosimetry.

sample	EPR signal, g-value	remarks
PVC floor plate	2.0056	not specific for irradiation, strong in unirradiated samples, after 1 year at 25°C irradiation not detectable, strong Mn^{2+}-signals.
PE-bag	2.0056	no signal in unirradiated samples, after 1 day at 25°C irradiation not detectable.
Nylon textile fibres	2.0056	not specific for irradiation, strong in unirradiated samples, after 1 year at 25°C irradiation not detectable.
Milk powder	2.0058	strong in unirradiated samples, not specific for irradiation, after 1 year at 25°C irradiation not detectable.
toilet detergent powder	2.0032	no signal in unirradiated samples, strong baseline drift, after 1 year at 25°C irradiation not detectable.

Participant 03: Doreen Stoneham

Accident dosimetry in populated areas: the use of solid state dosimetry techniques with ceramic and natural samples.

II Objectives for the reporting period (Stage 1)

1. Application and evaluation of standard thermoluminescent techniques for retrospective dose determination.
2. Selection of samples with low absorbed dose and poor fine-grain properties to see whether the signal and accuracy could be improved.
3. Extraction of quartz inclusions from these samples for TL measurements and for mineral characterisation.
4. Dose evaluation of high fired ceramics such as porcelain and stoneware tiles.
5. Dose-depth profiles for the different types of material.
6. An interlaboratory calibration.
7. Field trip to identify locations of suitable materials for further measurement.

III Progress achieved

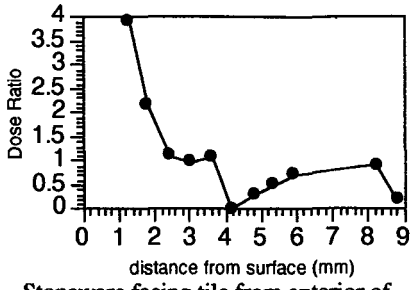
Dr Robert Adams was appointed in March to help with the experimental work. His previous training was as an experimental chemist and his doctoral thesis was on the extraction of radionuclides from low level radioactive waste. His contribution has been mineral extraction for inclusion measurements and preparation of high-fired ceramics for TL analysis.

The following objectives have been achieved:

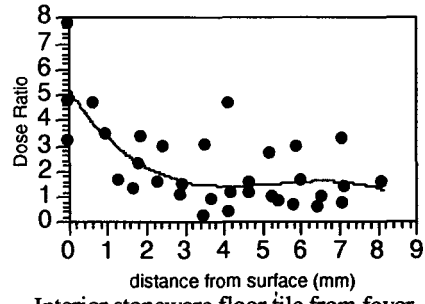
1. Thirteen samples were selected for TL measurements using both fine-grain and quartz inclusion techniques and their TL properties characterised. The samples included ten bricks of different origins and mineral composition and three high fired ceramics - a floor tile, an insulator and a porcelain bathroom fitting. The samples came from a range of locations both interior and exterior. Dose depth measurements were carried out on nine of the samples.

The results can be summarised as follows:
(i) Brick samples. The yield of quartz was very poor ranging from less than 1% to 9.4% from the bulk sample. Reproducibility of the dose estimates varied from 8% scatter to over 100% scatter. The latter result being obtained for one brick with an integrated dose of less than 0.03Gy. Most of the samples exhibited two peaks; one in the temperature range 210-230°C and the other in the range 350-360°C. Lower doses were obtained from the higher temperature peak for every sample and there was much higher scatter than for the low termperature peak. Three samples were also measured using the pre-dose technique and there was agreement between results using both pre-dose methods (Additive Dose and Multiple Activation). These broadly were in better agreement with high temperature results from the 210-230°C range. It was difficult to determine any definite dose depth trend due to the wide scatter of results, the disagreement between results from different temperature ordinates and the necessity of taking a large bulk sample (several mm thickness) in order to obtain sufficient yield of material for TL analysis.

(ii) High fired ceramics. These were sectioned into slices 9mm diameter by 200μm thick using a diamond blade and analysed using the pre-dose technique. After an initial TAC Sn-So was recorded and calibrated using a monitor dose of 0.75Gy. The aim was to obtain a dose-depth profile rather than absolute values at this stage. In addition it was possible to obtain data from the 150°C peak. In the case of the high-fired ceramics there was a definite dose-depth trend and it is intended to investigate this further. Two examples of dose-depth profiles are shown below.
A detailed report has been prepared for the Project.



Stoneware facing tile from exterior of electricity substation.



Interior stoneware floor tile from foyer of hospital.

2. A comparison of thick source alpha-counting versus neutron activation analysis (NAA) for the determination of the internal dose component.
The currrent work has concentrated on samples which had apparently absorbed a low dose and on methods of improving the accuracy of our results. One component of the total integrated dose is the internal dose component and when this is a large component of the total dose, it can make a correspondingly large contribution to the error term. This component can be determined in several ways. Two methods in common use are thick source alpha-counting combined with flame photometry, and NAA. In the past it has been observed that there has been some discrepancy between the two approaches and so we collaborated with Helsinki University to investigate this. It appears that the discrepancy arises from uranium chain either over-counting when using thick source alpha-counting or under-estimation of the activity when using NAA. A detailed report has been prepared for the project.

3. An interlaboratory calibration of the beta sources.
Samples of quartz which had been irradiated with 2.96Gy in a Co-60 beam by GSF were used to check the calibration of the standard beta sources in the Oxford laboratory's Riso automated TL readers. One reader had a previously calibrated $^{90}Sr/^{90}Y$ source and was found to be within less than 1% of the known GSF dose. A similar beta source in a second reader which had previously only been approximately calibrated was then calibrated directly using the same GSF quartz. A detailed report has been prepared for the project.

4. Fieldtrip.
A visit to Minsk (Institute of Radiobiology), Kiev (Institute of Radiation Medicine), Chernobyl (Checir and the External Dosimetry Control Department) and various locations in Byelorus and the Ukraine within the Exclusion Zone took place between July 22nd and August 5th. There was very successful collaboration with various groups, and visits were made to evacuated sites in Byelorus - Babchin, Radin, Masani, Lomachi,Tulgovichi and Solnichnaya and to evacuated villages in the Ukraine - Novoshepelichi, Starye Shapelichi, Kopachi, Burakovka and Tolstye Les as well as to Pripyat to survey potential sampling locations and apartment buildings on Lenin Prospect which are of interest to the modelling group at GSF. A lot of useful information was obtained regarding suitable sampling locations for a future field trip as well as information regarding the monitoring of such sites since the accident.

Publications relevant to the project:
Hutt G., Brodski L., Bailiff I.K., Goksu Y., Heaskell E., Jungner H. and Stoneham D. (1933) TL accident dosimetry using environmental material collected from regions downwind of Chernobyl: A preliminary evaluation. Radiat. Prot. Dosim. **47**, 307-311

Stoneham, D., Bailiff I.K., Brodski L., Goksu H.Y., Haskell E., Hutt G., Jungner H. and Nagatomo T. (1993) TL accident dosimetry measurements on samples from the town of Pripyat. Nucl. Trcks Radiat. Meas. **21**, 195-200.

Head of project 4: Dr. Bøtter-Jensen

II. Objectives for the reporting period

a) Developments of optically stimulated luminescence (OSL) techniques for dosimetry based on quartz and feldspars.
b) Investigation of OSL excitation and emission spectra for optimization of OSL dosimetry using quartz and feldspars.
c) Calibration of beta sources using TL and OSL of quartz.
d) OSL response of quartz to gamma radiation using the multiple aliquot technique.
e) OSL and TL responses of quartz to beta radiation using the single aliquot technique.
f) Dosimetry using quartz grains extracted from a modern brick.
g) Measurements of depth dose profiles in bricks exposed to different gamma photon spectra by means of TL and OSL.

Objectives for the next period:

- Further documentation of the above points.
- Evaluation of doses to brick and tile materials collected from the Chernobyl area using TL/OSL of quartz calibrated against Co-60 and Cs-137 reference doses provided at Risø.
- Evaluation of depth dose profiles in brick and tile materials to determine the absorbed dose utilising OSL measurements on mixed (unseparated) samples.
- Providing automatic scanning of brick cores using OSL for fast determination of depth dose profiles.
- Investigation of OSL sensitivity changes of quartz as a result of annealing bricks at high temperatures.
- Preparation of publications and final EC report

III. Progress achieved including publications

The following work was carried out at Risø National Laboratory under the contract **FI3PCT920040**. Two guest scientists, namely Dr. A Bluszcz, Silesian Technical University, Gliwice, Poland and Dr. H. Jungner, Helsinki University, Finland were assigned to the Risø project and they both contributed in achieving the results reported here.

Development of OSL techniques.
Equipment for measuring optically stimulated luminescence (OSL) of quartz using a filtered green wavelength band from a halogen lamp was recently developed at Risø (Bøtter-Jensen and Duller, 1992). The new OSL system, that also includes an infrared diode array for measuring feldspars, has shown an excellent stability and sensitivity in measuring low doses from quartz extracted from e.g. brick materials. Preliminary OSL measurements of fired quartz samples have shown that accummulated doses as low as 1 mGy can be determined with sufficient accuracy.

OSL excitation and emission characteristics.
A scanning monochromator design based on linearly variable interference filters has just been developed to be attached to

the above mentioned OSL unit (Bøtter-Jensen et al, 1993). The unit can be used for recording wavelength resolved luminescence excitation and emission spectra. The capabilities of the new scanning monochromator were tested and used to investigate the wavelength resolved luminescence excitation and emission spectra of a variety of quartz and feldspar materials suitable for dosimetry in the wavelength range 370 to 1020 nm (Bøtter-Jensen et al, 1993). Preliminary OSL studies of quartz samples have shown that the evaluation of dose apparantly is independent of the wavelength of the stimulation light in the visible range.

Dose rate calibration of beta source.
It was decided by the partners of the project to carry out intercomparative measurements of the strengths of the beta sources attached to their respective Risø TL/OSL readers. GSF provided standard samples of pure quartz which had received a gamma dose of 3 Gy. The calibration of a Sr/Y-90 beta source (5 mCi) at Risø was carried out using both TL and green light stimulated luminescence (GLSL) using the above mentioned equipment. The results are presented in Table 1. A systematic difference was found when determining the source strength by TL and GLSL, respectively and when using grains of different size. These results compare well with those reported by laboratories using quartz for TL and GLSL dating (Mejdahl and Bøtter-Jensen, 1993, Jungner and Bøtter-Jensen, 1993). A detailed calibration report was prepared as an internal Risø Report (Bluszcz and Bøtter-Jensen, 1993) and distributed to the partners. The Risø report is available on request.

Table 1. Results of the calibration of the beta source (5 mCi) using pre-irradiated pure quartz (3 Gy). TL: integration area 150-450°C, heating rate 8.33°C/s, detection filter Corning 7-59. GLSL: integration area 50 s, preheat 260°C/40 s, detection filter U-340.

	TL		GLSL	
Grain size (um)	100-150	150-250	100-150	150-250
Dose rate (mGy/s)	4.05±0.07	4.26±0.08	3.51±0.17	3.88±0.05

OSL dose response of quartz using a multi sample technique.
An attemp to determine the lower detection levels using TL and GLSL on fired quartz was made by obtaining dose versus TL/OSL response curves for a variety of quartz samples extracted from archaeological specimens (bricks and burnt clay). The samples were annealed at 500°C and irradiated in a calibrated Co-60 photon field kept in quartz/aluminium containers to ensure secondary electron equilibrium. The dose range was 0.1 to 4 mGy. The OSL response curve for a sensitive quartz extracted from burnt clay obtained using the multi-sample method is shown in Fig. 1A. As seen, the lowest detectable dose for this material is well below 1 mGy.

OSL and TL dose responses of quartz using a single aliquot technique.
A single aliquot method was also tested where the dose response (growth) curve was obtained using the regeneration technique. Beta irradiations were carried out within the Risø reader and GLSL measurements were carried out as described above. The results obtained show very little scatter because of the single aliqout technique where no normalisation is needed. Fig. 1B presents dose versus GLSL and TL response curves, respectively, in the dose range 0.1 to 5 mGy. The lower detection limit using GLSL on this particular quartz sample is seen to be well below 0.5 mGy and for TL below 1 mGy.

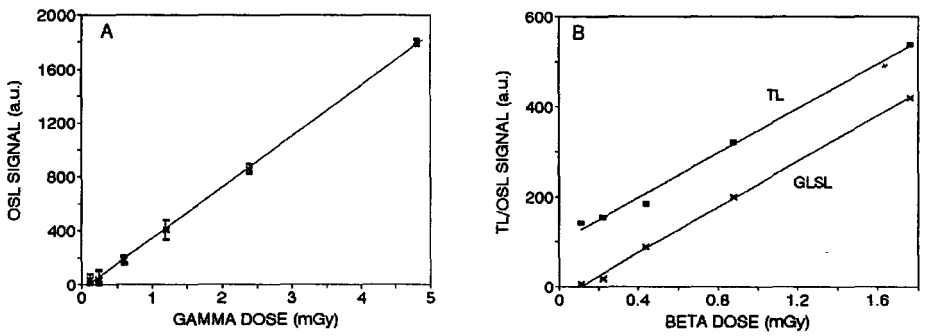


Fig.1.(A) OSL versus Co-60 gamma dose (multi sample technique) for quartz extracted from a burnt stone. (B) TL and OSL versus beta dose (single aliquot method) for the same quartz sample.

OSL dosimetry of natural quartz extracted from bricks.
The accumulated "natural" dose induced in quartz by radionuclides contained in modern brick materials was measured using GLSL on extracted quartz samples. The aim of this experiment was to determine the lower detection limit for an additional dose received by a brick as a result of radoactive release from a nuclear accident taking into account the GLSL contribution from the natural background dose. The brick was chosen from an outer wall of a laboratory building at Risø which was known to be about 40 years old. The quartz grains were extracted from the material and the absorbed dose was determined by GLSL using the additive dose technique. The result is shown in Fig. 2. The estimated dose is about 200 mGy which is in very good agreement with the expected value based on an annual dose rate of about 5 mGy/y from the natural radioactivity in the brick. For this particular brick a lower detection limit for an additional accidental dose would be in the order of 20 mGy (10% above the background).

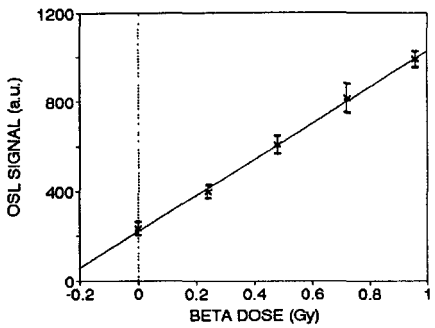


Fig.2. OSL versus added beta dose (additive dose technique) for quartz extracted from a 40 years old brick.

Evaluation of depth dose profiles in bricks

The aim of this experiment was to investigate the possibility of measuring depth dose profiles in irradiated bricks using unseparated (mixed) materials. Pieces from both modern and ancient bricks were annealed at 500°C to remove any previously acquired TL/OSL signal and then exposed to a dose of 5 Gy from Co-60 and Cs-137 photon radiation fields, respectively. After irradiation, 8 mm diameter cores were cut out of each piece of brick and sliced into 1 mm thick circular discs using a diamond saw. Each disc, representing a particular depth in the brick, had their GLSL directly measured from the surface of the unseparated material and as an example the OSL versus depth for an ancient brick irradiated to Co-60 radiation is shown in Fig. 3A. As seen, the half layer value is about 75 mm which corresponds reasonably well with the expected attenuation of Co-60 gamma photons in brick material. A brick collected from a house in the town Berezyaki in the Chernobyl area was further measured using the same procedure and the GLSL signal corresponding to the natural plus "accidental" dose versus depth is shown in Fig 3B. The more rapid attenuation seen here is in contrast to that obtained from the Co-60 irradiation and demonstrates that the Chernobyl brick was exposed to a gamma spectrum with a much higher content of low-energy photons.

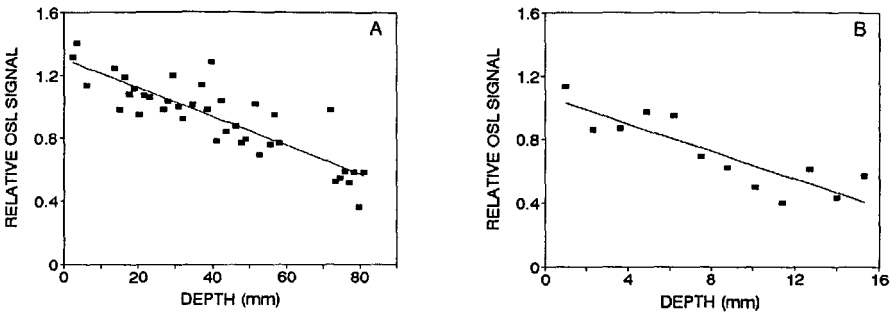


Fig.3.(A) OSL measured directly on unseparated fractions from an ancient brick irradiated to a 5 Gy Co-60 gamma dose after annealing at 500°C versus depth into the brick. (B) OSL versus depth measured directly on unseparated fractions for a brick collected at Chernobyl that had been exposed to an "accidental" dose.

New publications relevant for the project

Bøtter-Jensen,L and Duller,G.A.T.(1992) A new system for measuring OSL from quartz samples, Nucl.Tracks Radiat. Meas. **20** 549-553.

Bøtter-Jensen,L., Poolton,N.R.J., Willumsen,F. and Christiansen,H.(1993) A compact design for monochromatic OSL measurements in the wavelength range 380-1020 nm. Accepted for publication in Radiation Measurements.

Bøtter-Jensen,L., Duller,G.A.T. and Poolton,N.R.J.(1993) Excitation and emission spectrometry of stimulated luminescence from quartz and feldspars. Submitted for publication in Radiation Measurements (July 1993).

Bluszcz,A. and Bøtter-Jensen,L.(1993) Calibration of a beta source using TL and OSL on quartz. Risø Report No. I-725(EN).

Jungner,H. and Bøtter-Jensen,L. (1993) Study of sensitivity change of OSL signals from quartz and feldspars. Submitted for publication in Radiation Measurements (August 1993).

Mejdahl,V. and Bøtter-Jensen,L. (1993) Luminescence sensitivity changes of quartz and feldspar samples from archaeological materials and sediments when using the single aliquot/regeneration method. Submitted for publication in Ancient TL (March 1993)

Progress Report

Contract: **F13P-CT920064h** **Sector: A11**

Title: The measurement of the spectral and angular distribution of external radiations in the workplace and implications for personal dosimetry.

1) Clark NRPB
2) Perks UKAEA
3) Gualdrini ENEA
4) Chartier CEA - FAR

I. Summary of Project Global Objectives and Achievements

Objectives

The main objectives of the project are:

To investigate new methods of measuring the spectral and angular distribution of external radiations in the workplace, particularly for photons.

To improve current methods for measuring the spectra of neutron radiations in the workplace and prepare a compilation of measured neutron spectra.

To investigate the implications of spectral and angular distribution measurements for personal dosimetry, including methods of calibration of personal dosemeters and individual dose assessments in the workplace.

To improve methods of calculating photon and electron transport in phantoms, particularly the ICRU sphere and slab, to support practical calibration techniques for personal dosemeters.

To calculate backscatter factors for the ICRU sphere and to compare results with measurements in calibration laboratories.

To define procedures for calibrating personal dosemeters in terms of operational dose equivalent quantities in calibration laboratories.

To evaluate the performance of personal dosemeters (film and TLD) when measuring operational dose equivalent quantities in calibration beams.

I. (Continued)

Achievements

The main achievements of the project have been as follows:

To carry out basic measurements of photon radiations using a custom built spectrometer and to compare with predictions obtained using a Monte Carlo simulation code.

To complete a compilation of measured neutron spectra in the workplace and to improve techniques for measuring spectra.

To carry out a comprehensive set of Monte Carlo calculations of operational dose equivalent quantities in the ICRU sphere with reference to photon beams.

To implement calculation methods for effective dose quantities in anthropomorphine phantoms, for photon radiations.

To evaluate the ability of film and thermoluminescence dosemeters to measure personal dose equivalent $H_p(d)$ and establish a calibration method for this quantity using a 30 x 30 x 15 cm PMMA slab phantom.

Head of Project: Dr Clark

IIa. Objectives for the reporting period

i) To evaluate the performance of the NRPB custom built photon spectrometer, in particular its ability to measure energy and angular variations.

ii) To carry out measurements in simulated workplace spectra in the laboratory, thereby providing a check on the deconvolution methods used to analyse measured spectra.

IIb. Objectives for the next reporting period

The prime objective for the next reporting period is to build up a catalogue of measured workplace spectra and establish the full capabilities of the new spectrometer.

III. Progress achieved including publications

Preliminary measurements have been carried out using the spectrometer built at NRPB. The detector is a 17 mm x 25 mm sodium iodine crystal with a photomultiplier tube surrounded by lead shielding and a collimator. The size of crystal was chosen to minimise the amount of lead shielding required, and therefore make the spectrometer portable and easy to handle. This is an important consideration for practical workplace spectrometry where there may be problems of, for example, access or contamination. The schematic design of the spectrometer is shown in Figure 1 and a photograph of the complete assembly in Figure 2, the total weight being less than 15 kg.

Measurements have been carried out to facilitate the analysis of backscatter peaks (from the lead shielding), compton edge, characteristic x-rays, annihilation and full energy peaks. In particular the size of the lead shielding backscatter peak has been characterised using a shadow shield technique to eliminate potential effects from room scatter. A shield was placed close to the source (^{137}Cs and ^{60}Co) which attenuated the primary beam but has no significant effect on room scatter.

A series of test spectra have been developed to validate the unfolding programme and improve the ability of the spectrometer to measure energy and angular distributions of photon radiations. These spectra are intended to approximate common working environments, including calibration laboratories, industrial radiography facilities, nuclear power plants, hospital cancer therapy and diagnostic x-ray facilities.

Contact has been made with a number of organisations to make workplace measurements with the spectrometer. Contact has also been made with colleagues at Pacific Northwest Laboratories (Battelle) to exchange information on measurements carried out in the US. Some workplace spectral measurements have been carried out using larger, less portable, equipment capable of good energy spectra resolution, but no results have been published.

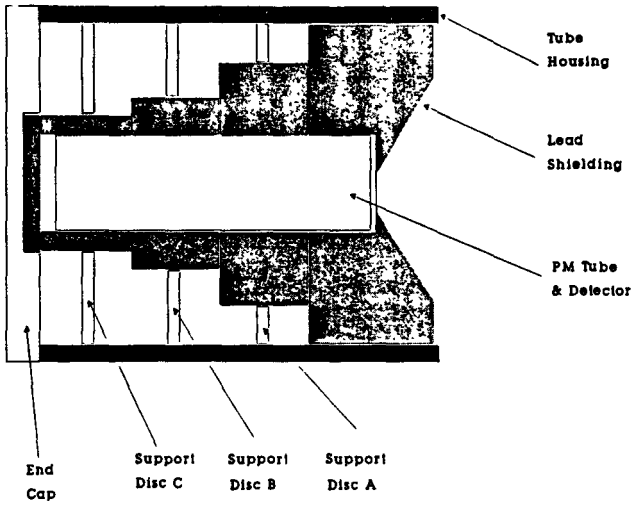


Figure 1. The outline design of the detector and shielding

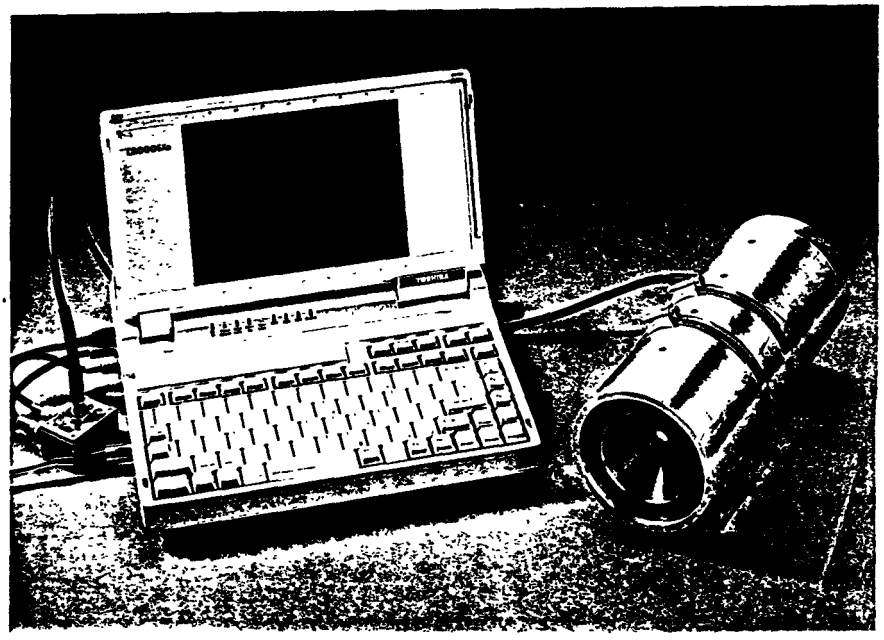


Figure 2. Photograph of complete assembly of the spectrometer

Head of project 2: Dr C A Perks

I. Objectives for the reporting period

(i) To develop the ESG4 Monte Carlo computer code to determine the response function matrix of the portable gamma-ray spectrometer, developed by NRPB, as a function of energy and angle.

(ii) To examine unfolding techniques developed for the portable gamma-ray spectrometer.

(iii) To prepare a compilation of neutron spectra measured with the Harwell neutron spectrometer system and assess the implications of these spectra for the calibration of personal dosemeters used in the workplace.

II. Progress achieved including publications

THEORETICAL SUPPORT FOR THE DEVELOPMENT OF A WORKPLACE GAMMA-RAY SPECTROMETER

The Monte Carlo computer model of the portable gamma-ray spectrometer (based on a shielded sodium iodide detector) developed by the NRPB has been further refined. In particular, variance reduction techniques (particle splitting and particle biasing) have been incorporated. These reduce run times for the calculation of response functions. A computer program has been written to introduce detector resolution, based on measurements made by the NRPB. Further comparison of calculated and measured spectra show good agreement. However, some time has been spent in trying to explain the rather large backscatter peak present in the measured source spectra compared to the calculated spectra. An increase in the size of the backscatter peak can be achieved in the computer model by using an extended source spectrum, but this is not able to fully explain the observed difference. In addition, all the expected features of gamma-ray spectra (full energy peaks, compton edge, escape peaks, annihilation peaks lead X-ray etc.) have been successfully identified in the calculated response functions.

We are currently producing a response function matrix for the portable gamma-ray spectrometer, and investigating the method for unfolding. It is anticipated that initial trials of the unfolding technique will be undertaken in late October.

NEUTRON SPECTROMETRY

Neutron spectrometry measurements were made at the ASPIS facility of the NESTOR Reactor at AEA Technology, Winfrith. These measurements were made with both sets of recoil counters (small spherical counters and large volume cylindrical counters) and the multisphere spectrometer and formed part of an intercomparison of spectrometry systems being organised by EURADOS Working Group 7. The results are currently being analysed and will be presented to the next EURADOS Working Group 7 meeting to be held in September.

A draft compendium of neutron spectra measured with proton recoil counters and a multisphere spectrometer has been written. This includes previous measurements of neutron spectra made at nuclear power stations (Gosgen, Switzerland, 2 locations; Dungeness 'A', UK, 3 locations; Hinkley Point, UK, 2 locations; and Trawsfynydd, UK, 1 location) Derived dosemeter responses are given, and comparisons of the dose-equivalent using new and old conversion factors are given. The draft compendium will be revised before publication to include the recent results from SILENE (reference measurements for the international intercomparison of criticality dosemeter systems) ASPIS and measurements of intermediate energy neutron beams. It will also include older measurements of the spectra around the DIDO and PLUTO reactors previously operated at Harwell.

OTHER

The significance of the angular and energy distribution of workplace radiation fields on radiation quantities defined by ICRU and ICRP were reviewed for the Workshop on Individual Monitoring of Ionising Radiation: The Impact of Recent ICRP and ICRU Publications held in Villigen, May 1993. The main conclusions of this paper were that for photons, the ratio of effective dose equivalent to effective dose lies in the range from 0.9 to 1.0 for practical spectra with primary energies greater than 80 keV. For workplace neutron fields, the new the new quantity of effective dose is greater than the old limiting quantity by factors of 1.5 to 5, but H*(10) will remain a reasonable overestimate for area monitoring. For individual monitoring, there are potential problems in that the operational quantity is likely to be less than effective dose for low energy spectra for irradiation conditions other than anterior-posterior.

PUBLICATIONS

Neutron Spectrometry and Dosimetry Measurements made at Nuclear Power Stations with Derived Dosemeter Responses', H J Delafield and C A Perks. In Proc. 7th Symp. on Neutron Dosimetry, Berlin, 14-18 October 1991. Radiation Protection Dosimetry, **44**, (1/4) pp227 -232 (1992).

Radiation Quantities: Significance of the Angular and Energy Distribution of the Radiation Fields, M Marshall, D J Thomas, C A Perks and O F Naismith. Paper presented to Workshop on Individual Monitoring of Ionising Radiation: The Impact of Recent ICRP and ICRU Publications held in Villigen, May 1993.

Overview of Compendium of Neutron Spectra measured for Radiological Protection Purposes. H J Delafield and C A Perks. Abstract submitted for IRPA Regional Congress on Radiological Protection, Portsmouth, June 1994.

Development of Energy and Angular Response Functions and an Unfolding Procedure for a Portable Gamma-ray Spectrometer. D Spencer, C A Perks, P Burgess and M Marshall. Abstract submitted for IRPA Regional Congress on Radiological Protection, Portsmouth, June 1994.

Head of project 3: Dr. Gualdrini

Object for the reporting period:

Calculations of directional dose equivalents in the ICRU sphere, comparison with data from measurements and literature.

Implementation of the anthropomorphic phantom ADAM in the Monte Carlo code MCNP for the evaluation of effective dose in workplaces.

Progress achieved including publications:

The Monte Carlo code MCNP has been employed /1/ to evaluate the quantity directional dose equivalent H'(d,ω) for the ICRU sphere at the three depths 0.007, 0.3 and 1 cm.

The calculations were carried out for the complete B.I.P.M. Series as well as for the four I.S.O. Series (Narrow, High Air Kerma Rate, Low Air Kerma Rate and Wide), as experimentally obtained at the ENEA Primary Standard Dosimetry Laboratory/2/.

The sphere was subdivided into 455 cells using concentric spheres and cones, to create twelve angular sectors from 0 to 180 degrees. Radial steps of 0.14 mm (first layer), 2 mm until 1 cm and 5 mm for the rest of the sphere were used (figure 1).

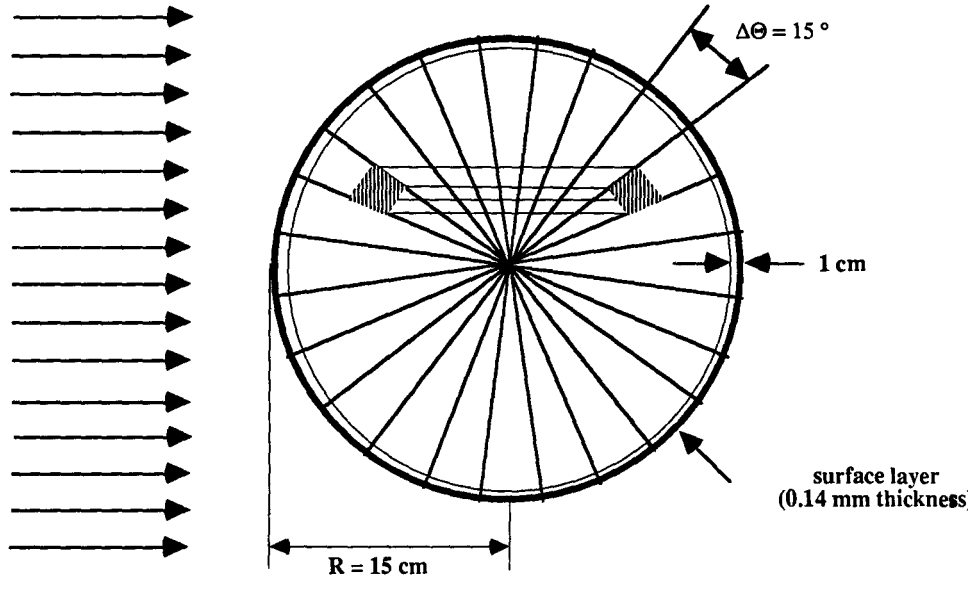


Fig. 1 : Simplified scheme of the ICRU sphere as described in the MCNP code for the calculation of directional dose equivalents.

The calculations have been carried out in the Kerma approximation, neglecting the secondary electron transport, whilst taking into account a detailed cross section treatment in the

lower energy domain (fluorescent emission and modification to the Klein-Nishina cross section due to electron binding effects, expressed through appropriate form factors).

The calculated quantity was the ratio between the $H'(d,\omega)$ and K_a , air collision Kerma at the same point with the phantom absent. The results were also compared with literature results, obtaining a satisfactory agreement (Tables I, II and III). An example of radial behaviour of the directional dose equivalent for the various incident angles is supplied in Figures 2 and 3 for the Wide Spectrum Series 60 kV and 80 kV beams.

Figures 4, 5 and 6 show the angular behaviour for the three depths versus the mean energy of the incident beam for the complete Wide Spectrum Series.

A series of experimental comparisons is underway using very small ionisation chambers (PW mod. M2332) in the tissue substitute RS-1 sphere /3/ at 10 mm depth. This activity has also been finalised to a validation of the code to extend the computational and experimental studies to the evaluation of the Hp at 10 mm in PMMA and RS-1 30X30X15 cm slabs for calibration purposes, investigating the energy domain among 20 keV to 10 MeV.

A second activity was addressed to the study of an anthropomorphic phantom for MCNP.

The ADAM phantom was translated into the MCNP input format in the framework of a collaboration with the KFA Julich Institute (Federal Republic of Germany).

The Sabrina Code was employed to graphically display the MCNP input and to test the geometry inconsistencies (in figures 7 and 8 two 3-dimensional colour plots are reported).

The MCNP Monte Carlo code will be employed to determine effective dose in specified workplace conditions taking into account, besides the particle spectrum, also the irradiation history and conditions of the individual exposed to the investigated radiation field. These workplace data will be supplied by NRPB and AEA Laboratories (UK).

Publications: Morelli B., Gualdrini G.F., Monteventi F. 'Field Parameters and Operational Quantities for the ICRU sphere with Reference Photon Beams : PART 4 Monte Carlo and Experimental Evaluation of Angular Dependence at Specified Depth Dose Equivalent Quantities. RT/ENEA/AMB (In press)

References:

/1/ Morelli B., Gualdrini G.F., Monteventi F. ,"Field Parameters and Operational Quantities for the ICRU sphere with Reference Photon Beams : PART 4 Monte Carlo and Experimental Evaluation of Angular Dependence at Specified Depth Dose Equivalent Quantities." RT/ENEA/AMB (In press)

/2/F. Laitano et al., " Energy Distributions and Air Kerma Rates of ISO and B.I.P.M. Reference Filtered X-Radiation ", ENEA Report, December 1990.

/3/ K. P. Hermann, D . Harder, "Production and Use of a Polyethylene-Based Tissue-Equivalent Spherical Phantom Substituting the ICRU sphere for Photons." Rad Prot Dosim Vol 28 (1989)

TAB. I : H'(0.07)/ka conversion coefficiens for the various B.I.P.M. and ISO spectra.

Series (*)	Tube Voltage (kV)	ENEA	PSD	Grosswendt /3/	Wagner /4/	ICRP /5/	Ratio ENEA/Grosswendt
P1	10	0.90	0.7				
P2	25	0.99	0.9				
P3	30	0.99	0.9				
P4	50	1.11	1				
P5	50	1.25	1				
P6	100	1.39	1				
P7	135	1.55	1				
P8	180	1.56	1				
P9	250	1.45	1				
A2	10	0.88	0.7	0.90	0.90	0.90	0.98
A3	20	0.96	0.8	0.97	0.97	0.96	0.99
A4	30	1.02	1	1.05	1.05	1.03	0.97
A5	60	1.28	1	1.36	1.34	1.32	0.94
A6	100	1.51	1	1.54	1.53	1.51	0.98
A7	200	1.53	1	1.54	1.53	1.53	0.99
A8	250	1.45	1	1.50	1.49	1.50	0.96
A9	280	1.44	1	1.45	1.45	1.46	0.99
A10	300	1.42	1	1.46	1.45	1.46	0.97
L1	60	1.44	1	1.47	1.46	1.44	0.98
L2	80	1.55	1	1.57	1.55	1.54	0.99
L3	110	1.61	1	1.60	1.59	1.58	1.01
L4	150	1.53	1	1.54	1.54	1.53	0.99
L5	200	1.45	1	1.46	1.46	1.46	0.99
L6	250	1.39	1	1.40	1.39	1.41	0.99
L7	300	1.36	1	1.36	1.35	1.37	1.00
S1	40	1.27	1	1.29	1.27	1.24	0.98
S2	60	1.50	1	1.51	1.49	1.47	0.99
S3	80	1.62	1	1.62	1.59	1.58	1.00
S4	100	1.62	1	1.60	1.59	1.58	1.01
S5	120	1.56	1	1.54	1.55	1.55	1.01
S6	150	1.49	1	1.50	1.50	1.50	0.99
S7	200	1.41	1	1.41	1.40	1.41	1.00
S8	250	1.36	1	1.35	1.34	1.37	1.01
S9	300	1.32	1	1.31	1.32	1.33	1.01
B1	35	1.22	1	1.24	1.22	1.19	0.98
B2	55	1.51	1	1.51	1.50	1.48	1.00
B3	70	1.61	1	1.61	1.58	1.57	1.00
B4	100	1.61	1	1.59	1.59	1.58	1.01
B5	125	1.52	1	1.52	1.53	1.53	1.00
B6	170	1.43	1	1.44	1.43	1.44	0.99
B7	210	1.38	1	1.38	1.36	1.39	1.00
B8	240	1.35	1	1.35	1.34	1.37	1.00

(*)
P: B.I.P.M. Series
A: High Air Kerma Rate Series
L: Wide Spectrum Series
S: Narrow Spectrum Series
B: Low Air Kerma Rate Series.

TAB. II : H'(3)/ka conversion ceofficiens for the various B.I.P.M and ISO spectra.

Series (*)	Tube Voltage (kV)	ENEA	PSD
P1	10	0.064	2
P2	25	0.61	1
P3	30	0.52	1
P4	50	1.01	1
P5	50	1.25	1
P6	100	1.44	1
P7	135	1.60	1
P8	180	1.62	1
P9	250	1.51	1
A2	10	0.046	2
A3	20	0.37	1
A4	30	0.72	1
A5	60	1.26	1
A6	100	1.57	1
A7	200	1.58	1
A8	250	1.51	1
A9	280	1.48	1
A10	300	1.45	1
L1	60	1.51	1
L2	80	1.63	1
L3	110	1.68	1
L4	150	1.59	1
L5	200	1.49	1
L6	250	1.43	1
L7	300	1.39	1
S1	40	1.27	1
S2	60	1.56	1.3
S3	80	1.70	1.3
S4	100	1.69	1.3
S5	120	1.62	1.2
S6	150	1.55	1.2
S7	200	1.45	1
S8	250	1.39	1
S9	300	1.34	1
B1	35	1.22	1
B2	55	1.58	1.3
B3	70	1.68	1.3
B4	100	1.67	1.3
B5	125	1.58	1.2
B6	170	1.47	1.2
B7	210	1.41	1.2
B8	240	1.38	1.2

(*)
P: B.I.P.M. Series
A: High Air Kerma Rate Series
L: Wide Spectrum Series
S: Narrow Spectrum Series
B: Low Air Kerma Rate Series.

TAB:III : H'(10)/ka conversion coefficiens for the various B.I.P.M. and ISO spectra.

Series (*)	Tube Voltage (kV)	ENEA	PSD	Grosswendt /3/	Wagner /4/	ICRP /5/	Ratio ENEA/Grosswendt
P1	10	0.003	28				
P2	25	0.27	1.5				
P3	30	0.22	1.6				
P4	50	0.75	1.5				
P5	50	1.12	1.5				
P6	100	1.40	1.5				
P7	135	1.67	1.5				
P8	180	1.67	1.4				
P9	250	1.53	1.4				
A2	10	-		-	-	-	
A3	20	0.09	2	0.20	0.20	0.17	0.45
A4	30	0.4	2	0.57	0.57	0.56	0.70
A5	60	1.15	2	1.30	1.32	1.29	0.88
A6	100	1.60	2	1.63	1.63	1.62	0.98
A7	200	1.64	1	1.63	1.64	1.64	1.01
A8	250	1.54	1	1.58	1.59	1.59	0.97
A9	280	1.47	1	1.52	1.53	1.52	0.96
A10	300	1.46	1	1.52	1.53	1.53	0.96
L1	60	1.51	1.5	1.52	1.54	1.51	0.99
L2	80	1.71	1.5	1.67	1.68	1.66	1.02
L3	110	1.77	1.4	1.71	1.71	1.71	1.03
L4	150	1.66	1.4	1.62	1.63	1.64	1.02
L5	200	1.51	1.4	1.52	1.54	1.54	0.99
L6	250	1.44	1.3	1.45	1.46	1.45	0.99
L7	300	1.38	1.3	1.40	1.41	1.39	0.98
S1	40	1.19	1.5	1.19	1.20	1.18	1.00
S2	60	1.60	1.5	1.58	1.60	1.57	1.01
S3	80	1.78	1.5	1.74	1.74	1.73	1.02
S4	100	1.75	1.4	1.70	1.71	1.72	1.03
S5	120	1.67	1.4	1.64	1.65	1.65	1.02
S6	150	1.59	1.4	1.57	1.59	1.59	1.01
S7	200	1.45	1.3	1.46	1.47	1.46	0.99
S8	250	1.37	1.3	1.40	1.40	1.38	0.98
S9	300	1.32	1.3	1.36	1.35	1.34	0.97
B1	35	1.11	1.4	1.09	1.10	1.09	1.02
B2	55	1.63	1.5	1.59	1.61	1.57	1.025
B3	70	1.77	1.4	1.73	1.73	1.72	1.02
B4	100	1.72	1.4	1.69	1.70	1.71	1.02
B5	125	1.63	1.4	1.60	1.62	1.62	1.02
B6	170	1.50	1.3	1.49	1.51	1.51	1.01
B7	210	1.41	1.3	1.42	1.43	1.41	0.99
B8	240	1.36	1.3	1.39	1.39	1.37	0.98

(*)
P: B.I.P.M. Series
A: High Air Kerma Rate Series
L: Wide Spectrum Series
S: Narrow Spectrum Series
B: Low Air Kerma Rate Series.

Fig.2 : Wide Spectrum Series (60 kV)

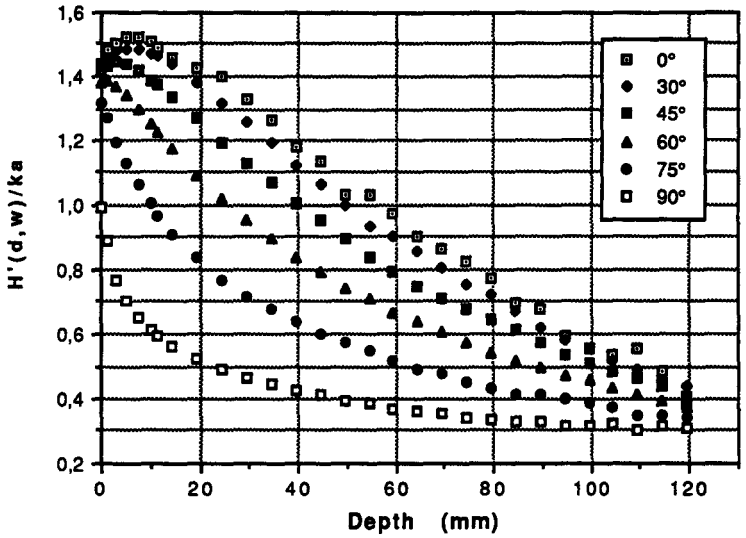
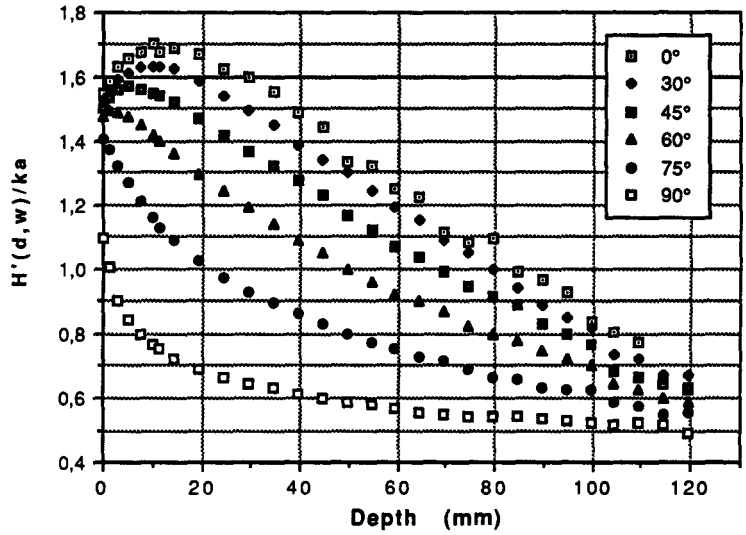


Fig.3 : Wide Spectrum Series (80 kV)



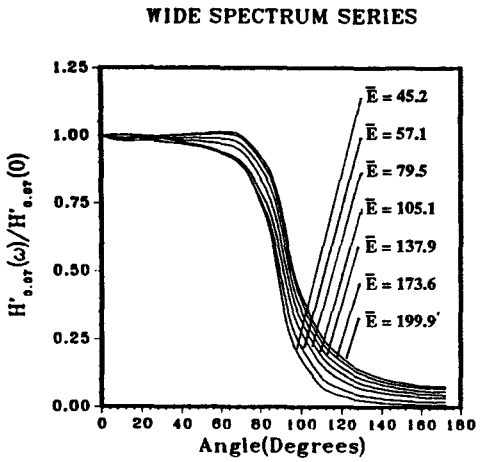


Fig. 4 : $H'_{0.07}(\omega)/H'_{0.07}(0)$ dependence on the radiation incidence direction for different photon energies.

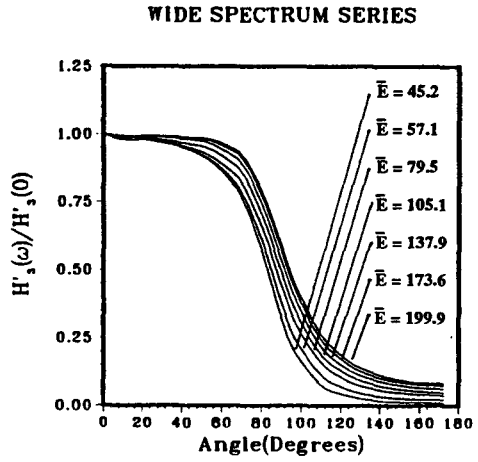


Fig. 5 : $H'_3(\omega)/H'_3(0)$ dependence on the radiation incidence direction for different photon energies.

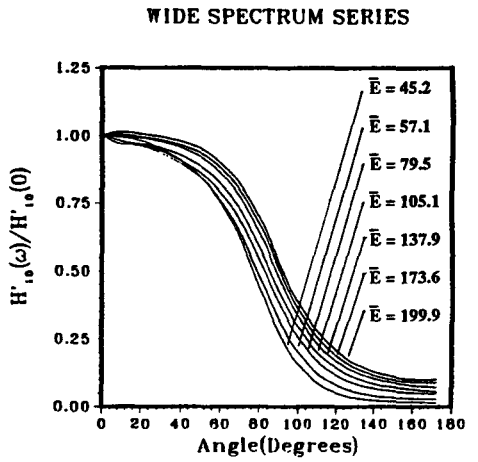


Fig. 6 : $H'_{10}(\omega)/H'_{10}(0)$ dependence on the radiation incidence direction for different photon energies.

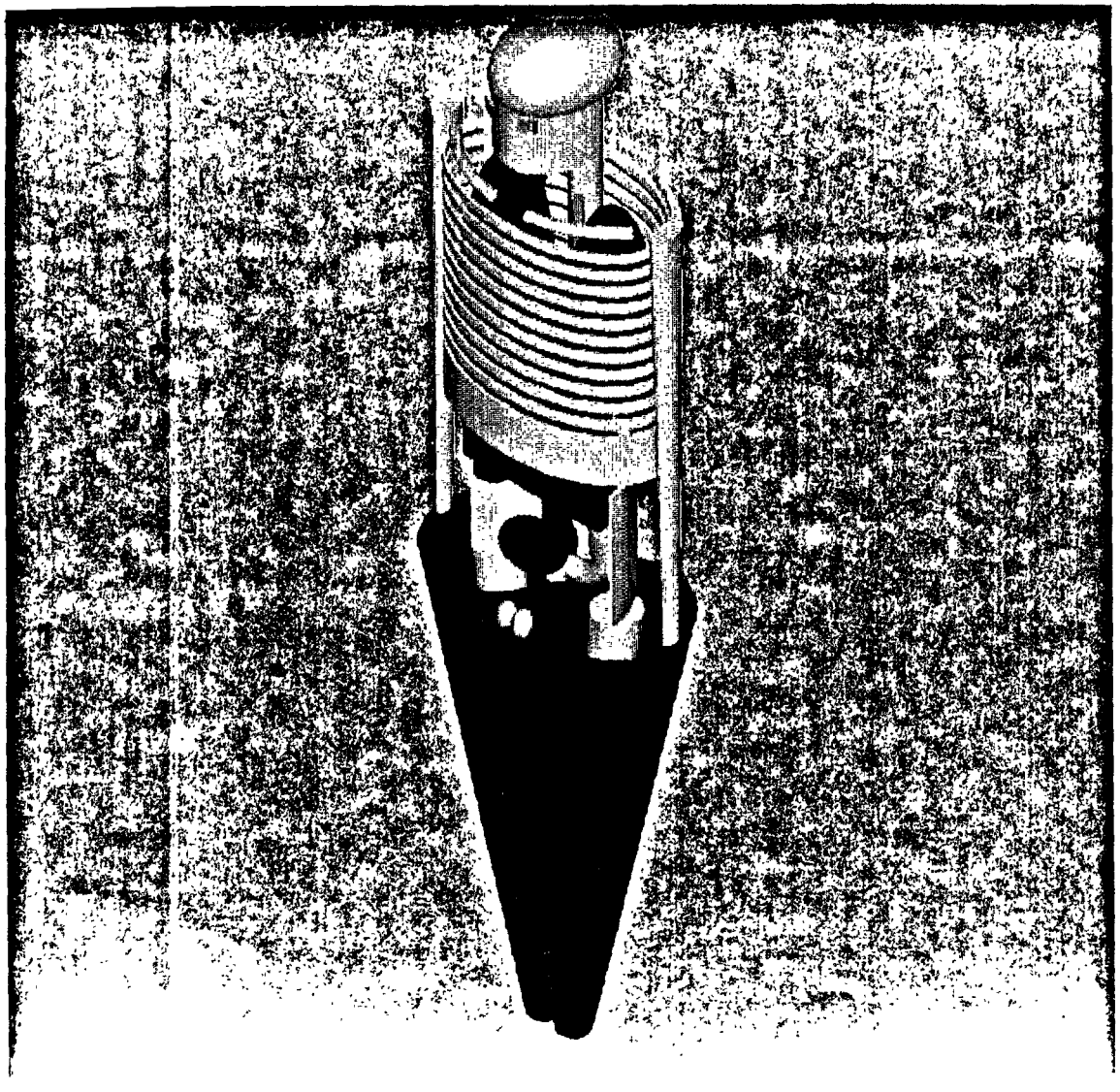


Figure 7 :

3 dimensional colour plot of internal organs of the ADAM phantom as produced by the code SABRINA (MCNP geometry)

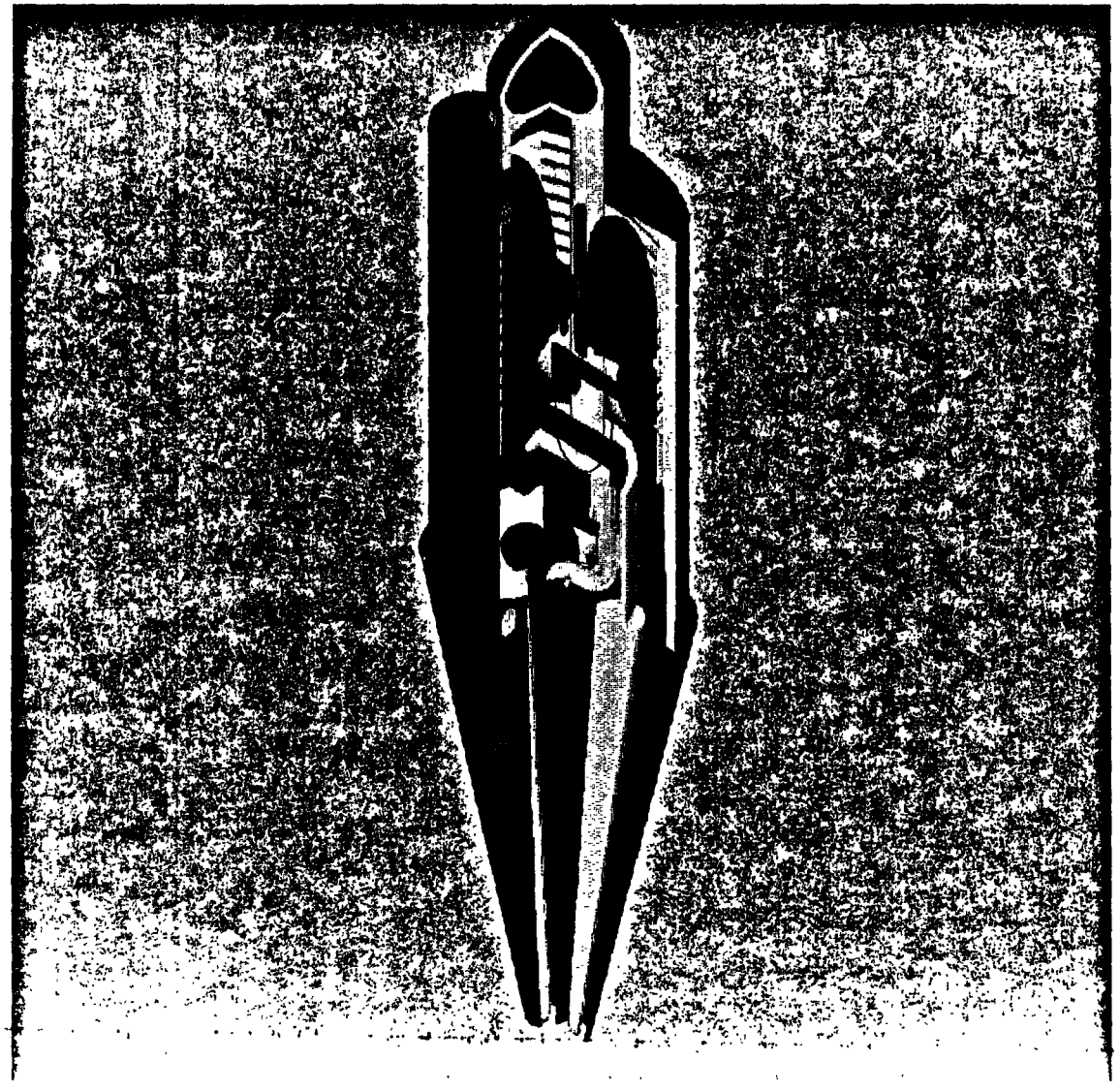


Figure 8 :

3 dimensional colour plot of internal organs of the ADAM phantom as obtained by the SABRINA code using two vertical cutting plane surfaces (MCNP geometry)

Head of project 4: Dr. Chartier
Scientific staff : C.Itié, D.Cutarella

II. Objectives for the reporting period

Considering the date at which the administrative procedures have been completed and official approval by authorities expressed,the content of this progress report will be inevitably limited. The CEA/SDOS contribution deals mainly with experimentations involving irradiations of individual dosemeters in photon radiation beams. Experimental conditions are progressively modified to replicate, as closely as possible, some characteristics of practical fields as those measured by AEA Harwell and NRPB. Equipments have been purchased to perform the simulation of "realistic" irradiation conditions and preliminary tests of new photographic emulsions had to be performed before continuing the evaluation of dosemeters currently in operation at CEA Fontenay-aux-roses.

II.A. Objectives for the next reporting period

In the next period, the program will include on-phantom irradiations of individual dosemeters as recommended in ICRU Report 47 to implement the dose equivalent operational quantities (ICRU Report 39). The final objective of the programme is to provide a dosimetry service with a procedure enabling to take advantage of the knowledge of the photon spectrum encountered in the vicinity of a nuclear installation. The methods of replicating a "practical" photon spectrum will be considered in order to take into account the energy and angle distributions combined with the movement of the dosemeter in the radiation field.

III. Progress achieved during the reporting period.

The reporting period has been exclusively used by the staff of CEA/SDOS to prepare and organise a programme of experimentations and calculations. This programme will be presented and discussed at the first meeting of the Joint Contract (scheduled in October 93 at CEA Fontenay), then brought into operation and completed for the end of the contract (June 94). To give more weight to the conclusions which are expected to derive from the planned programme, a contribution will be asked to the other contractors, including mainly the irradiation of dosemeters routinely used in their own country.

The current content of the CEA/SDOS involves basically series of irradiations of individual dosemeters. The conditions are described hereafter :

1° - Determination of the energy dependence and the angle dependence of the dosemeter.
On-phantom irradiations should be perform as recommended in ICRU Report 47 (PMMA slab phantom) and according to procedures given in ISO Standards. Similar irradiations previously performed have

shown that a batch of 5 dosemeters is adequate to characterise 1 "point" (understood : energy and angle of incidence of the radiation beam).

2° - Simulation in the laboratory of a "realistic" photon spectrum.
The word "realistic" applies to a radiation field encountered in the vicinity of a nuclear installation. It implies a "wide" spectrum and an angular distribution as those which should be measured with the NaI spectrometer currently in progress at Harwell and NRPB laboratories. Assuming that such data are available, a software should enable to replicate such energy distribution from a weighted combination of ISO reference radiations.

3° - Calculation of the dosimetric quantity Hp(10).
The knowledge of the characteristics of the replicated photon spectrum enables to calculate the reference value when irradiations are performed with such radiation qualities. It is intended to compare 2 evaluations of the ratio between the calculated value of Hp(10) and the indication I of the dosemeter (i.e. the calibration factor). In the first method, the "calculated" indication derived from the contributions of the components of the replicated spectrum, whereas in the 2nd method, the real reading (or indication) should be taken into account. The quality of agreement between both values will provide elements to verify that the knowledge of the photon spectrum enables to improve the degree of accuracy of dosimetry with individual devices.

4° - Another step to pass through is to take into account the movement of a person with its dosemeter in the radiation field. This situation should be approximated by a computer-controlled movement of the irradiation phantom during the irradiation process.

5° - To infer the most significant conclusions from this work, it is essential to enlarge the programme (or a part of) to several types of dosemeters : photographic films, TLD dosemeters .. and for each type, to include samples operated in different countries. The contribution of other calibration laboratories should be asked for.

Progress Report

Contract: FI3P-CT920002 Sector: A12

Title: Realistic neutron calibration fields and related dosimetric quantities.

1)	Klein	PTB
2)	Thomas	NPL
3)	Chartier	CEA - FAR
4)	Schraube	GSF

I. Summary of Project Global Objectives and Achievements

The main objective of this collaborative project is to provide in the laboratory a few well-characterized neutron fields that replicate typical spectral neutron fluence distributions met in radiation protection practice. The project consists of four distinct parts, namely:

a) the measurement of typical spectral neutron fluences encountered in practical situations,

b) the preparation of a catalogue of measured neutron spectra, response functions of commonly used neutron detectors and dosemeters, and various fluence-to-dose conversion functions,

c) the development of a program system for handling this catalogue, e.g. to calculate dosimetric quantities of the fields, to estimate the readings of detector systems and systematically to inspect the catalogue for a few basic spectra and

d) the computational prediction of configurations consisting of easily accessible neutron sources and appropriate moderators to realize these basic fields in the laboratory for calibration purposes.

The four collaborating laboratories at CEA, GSF, NPL and PTB contributed to different topics and progress was achieved in all parts, namely:

a) All laboratories succeeded in improving their neutron spectrometers used in the field. Most remarkable is the quality achieved in calculating the response functions of the different Bonner sphere spectrometers used. The MCNP predictions on the basis of realistic detector models are in excellent agreement with calibration data except for an overall normalisation factor which, however, is independent on the neutron energy and the diameter of the spheres. The spectral neutron fluence obtained by unfolding on the basis of the new response matrices will now be more reliable than previous data although a rigorous uncertainty propagation is still missing.

GSF, NPL and PTB participated in comparison excercises of spectrometers and dosemeters, initiated and partially organised by EURADOS WG7. The Swedish Radiation Protection Institute offered the possibility to measure in the containment of a pressurized water reactor and in the environment of a transport container for used fuel elements. The comprehensive set of data obtained with different neutron spectrometers, TEPC systems, survey instruments and personal dosimeters is being evaluated. The Winfrith Technology Centre prepared two different fields at the ASPIS facility of the NESTOR test reactor to be at first investigated with neutron

spectrometers. The measurements were performed in spring 1993 and the resulting spectral fluences must still be evaluated.

b) At GSF and PTB a considerable effort was spent to calculate fluence to dose conversion functions taking into account recent recommendations of ICRP (publ. #60). The new conversion functions are included in the catalogue and were already used at NPL to study their influence on the dosimetric quantities of typical neutron fields and the reading of commonly used monitors. These results were presented at a workshop in Villigen.

c) The catalogue was continuously updated and a manual of the program package SPKTBIB has been drafted. The first version of the system, i.e. the catalogue and the handling program, may be released by the end of the year to be tested by interested experts involved in radiation protection practice.

d) The MCNP calculations of the moderator assembly realized at the 14 MeV generator of CEA in Cadarache has been refined. The calculations are in satisfying agreement with the experimental data already reported. Another realistic calibration field has been designed to be realised with a 2.8 MeV neutron generator also available at CEA in Cadarache.

Head of project 1 : Dr. H. Klein

Scientific staff: A. Alevra, Dr. K. Knauf, Dr. B.R.L. Siebert, Dr. B. Wiegel, J. Wittstock

II.1 Objectives for the reporting period

The main objectives were :

a. to calculate the response matrix of the PTB Bonner Spheres C system with the MCNP code and a realistic detector model,
b. to measure at working places and in calibration fields,
c. to calculate fluence-to-dose (equivalent) conversion functions and
d. to update and inspect the new catalogue.

II.2 Objectives for the next reporting period

The program comprises :

a. to establish an improved response matrix of the PTB BS systems,
b. to measure in the vicinity of a transport container with ^{252}Cf neutron sources both free in air and in a salt mine,
c. to update the catalogue and to release the first version of the handling program for experts and
d. to calculate moderator assemblies with accelerator based neutron sources suited to replicate typical neutron fields encountered at working places.

III. Progress achieved including publications

III.1. MCNP Calculations of the Bonner Sphere Spectrometer Response in Comparison with Calibration Data.

One of the main purposes of the calculation of Bonner sphere (BS) response functions is to obtain data for the whole range of possible neutron energies without the gaps as they are present for experiments with monoenergetic neutron beams [1]. The BS spectrometer dealt with in this report is the socalled PTB C set [1]. It consists of an SP90 ^3He-filled spherical proportional counter as neutron detector and a set of 12 polyethylene (PE) spheres ranging from 7.6 cm (3") to 45.7 cm (18") in diameter. The absolute values of the calculated response functions $R_d(E)$ are adjusted to the experimental calibration points by introducing adjustment factors which are determined by performing a least square fit to all available experimental energies [2] for a certain sphere diameter.

Figure 1 shows the results for the calculations using the ANISN code [3] and the Monte Carlo Neutron and Photon (MCNP) transport code [4]. The variation of these fit coefficients relative to the value 1 range from -15% to +11% and -5% to +11% for the ANISN(NPL) and the MCNP(GSF) calculations, respectively. Especially a drop of the fit factors for small BS's ($d \leq 5$") is obvious. Thus we decided to take greater care of the geometry and the materials used in the detector. The main improvements of the model are the realistic description of:

- the 3-dimensional geometry of the "nose" and the "stem" of the detector which are partially filled with ^3He and air, and
- the internal structure of the detector.

The information needed was extracted from a technical drawing of the SP90 counter provided by the manufacturer [5]. The detailed geometry input for MCNP will be soon published. Here we note that the value used for the ^3He number density is $n = 4.94 \cdot 10^{19}$ atoms/cm^3 ($p = 200$ kPa) and for the PE density $\rho(PE) = 0.946$ g/cm^3. The fit coefficients are each adjusted to calibration data at up to 13 neutron energies (thermal and 1.17 keV to

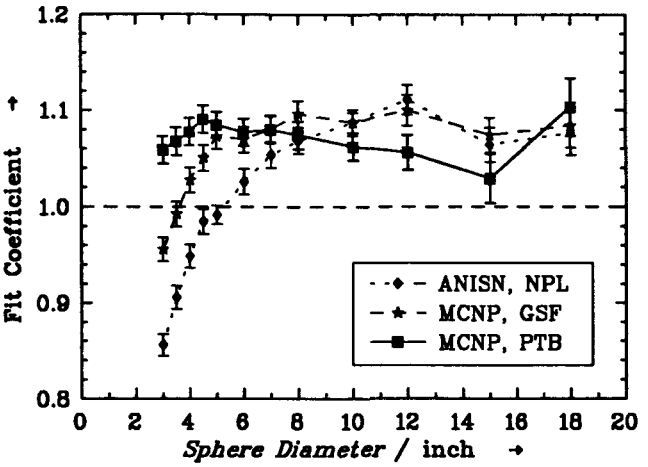


Fig. 1: Fit coefficients to adjust three different sets of calculated Bonner sphere responses [3,4,this work] to experimental data [1].

14.8 MeV). The results of the MCNP calculation using the realistic geometry are also shown in Fig. 1. They vary from +3% to +11% and do not show the same trend for small spheres.

Up to date we have calculated a total of 918 response values, 76 for the bare detector between 1 meV and 1 keV with relative statistical uncertainties (given by the MCNP program) of less than 0.2% and 842 values for the 12 spheres of the C system between 1 MeV and 20 MeV. Their relative uncertainties for the sensitive regions are less than 0.5% for the small BS's ($d \leq 6"$), about 1% for the 12" BS and less than 2% for the 18" BS.

To qualitatively demonstrate the results of MCNP calculations with the improved geometry, Fig. 2 shows the response functions for 3", 5" and 10" BS's as thick lines. The lines are straight connections of calculated data. Not to overload the figure they are not plotted as symbols. For comparison the histograms represent the calculations with the ANISN code [3]. All calculated response functions (MCNP and ANISN) have been separately adjusted to fit the experimental data [1] which are also included in Fig. 2. For neutron energies above 10 eV both calculations fit well the experimental data. The realistic treatment with MCNP also fits the values measured at thermal energies. There is a major disagreement for the ANISN data [3] which overestimate the measurements by a factor of 1.5 to 2. The good aggreement of measurements and MCNP calculations over the entire energy range and all moderator diameters support the assumption that the new model is a realistic description of the experiment.

Additionally we have calculated the response function for the 12" BS in more detail at 79 energies between 2 and 10 MeV as shown in Fig. 3. The statistical uncertainties are given by the height of the symbols, the thick line is their straight connection. For comparison the cross section density of polythylene $\Sigma_{PE}(E)$ is also shown. While the hydrogen cross section is a smooth function here structures arise due to resonances of the carbon cross section. At such a resonance energies (e.g. E_n = 2.077 MeV) there is an increased probability that the interactions of the neutron with the PE moderator are collisions with a carbon nuclei instead of hydrogen. The neutrons may leave the moderator without being thermalized. This results in a pronounced dip of the

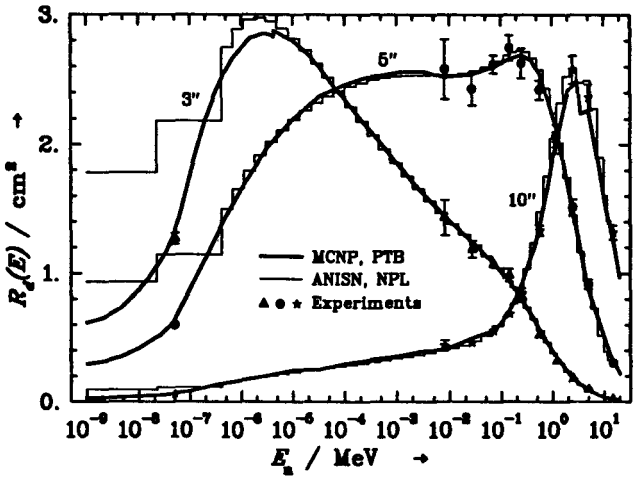


Fig. 2: Comparison of calculated responses $R_d(E)$ using the MCNP code with the improved model of the SP90 counter, results from the ANISN code [3] and experimental data [1] for three selected sphere diameters.

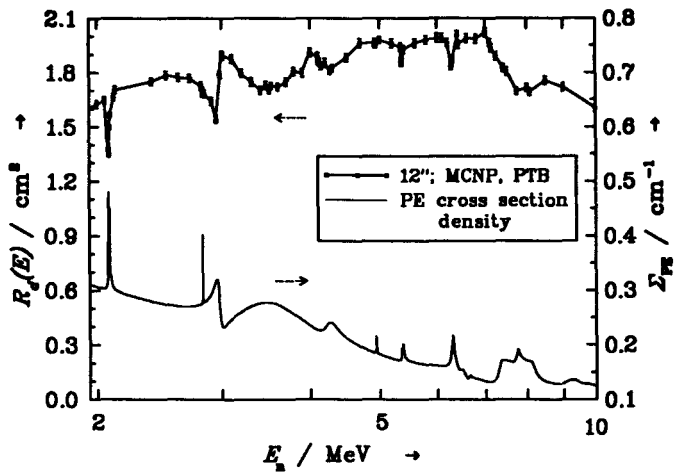


Fig. 3: Response function $R_d(E)$ of a 12" Bonner sphere (left axis) for neutron energies between 2 and 10 MeV and the neutron cross section density function of polyethylene $\Sigma_{PE}(E)$ (right axis) in the same energy window.

response at that energy. The next task will be to check this structure, also for other sphere diameters and to look for a generalized description.

III.2 Measurements at working places

Under the auspice of EURADOS WG7 the Swedish Radiation Protection Institute (SSI) invited various laboratories to measure in the containment of a pressurized water nuclear power reactor and in the vicinity of a transport container with used fuel elements. Seventeen groups from seven countries took part with neutron spectrometers, TEPC's, survey instruments and personal dosimeters. The measurements were performed at Ringhalsverket and Oskarshamn in November 1992. The PTB employed the BS system and a Leake type area dosimeter at five different positions. The unfolded spectral neutron fluences were submitted for an evaluation, which still has to be discussed among all participants. Although the measuring conditions were extremely hard with regard to the environmental conditions (temperature, humidity, acustic noise) and restrictions in the measuring time, the results seem to be reliable. Total neutron fluences and dose equivalents were evaluated with uncertainties of $< 5\%$ and 10% resp.. A comprehensive report is in preparation comparing the results of all different techniques employed.

In addition, the neutron fields used at the Nuclear Research Center in Karlsruhe for the calibration of area and personal dosimeters (in particular Albedo dosemeters) were investigated with the BS spectrometer. The resulting neutron spectral fluences confirmed the supposition that with increasing distance to the neutron source (bare Cf or Am/Be) the mean neutron energy considerably decreases. A comparison with other dosimetric data is in preparation.

III.3 Catalogue and conversion functions

An important application of the program handling the catalogue is to calculate mean fluence-to-dose (equivalent) conversion coefficients for the spectra collected in the catalogue.

New calculations of conversion coefficients for neutrons were necessary in order to follow the recommendations given by ICRP [6]. Several sets of conversion coefficients were published [7-11] and intercompared [12].

The work presented in Ref. [11] was in part supported by this project. The data for the ambient dose equivalent, $H^*(10)/\Phi$ as computed for Ref. [11] and collated from the literature [7-10,12] were evaluated in order to fit the coefficients of an analytical function. These fits cover the neutron energy range from thermal to 180 MeV, but the data beyond 30 MeV are based on one calculation only [10]. Additional work is required, because neutrons of this energy range chiefly contribute to the personal dose obtained in aircrafts flying at altitudes higher than 10 km and at high energy hadron accelerators.

The resulting functions for the ambient dose equivalent $H^*(10)/\Phi$ and the absorbed dose $D^*(10)/\Phi$ are now included in the SPKTBIB program and were used to study the relevance of the new ICRP recommendations for neutron fields encountered in radiation protection practice.

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Publications:

[11] B.R.L. Siebert, H. Schuhmacher, Calculated fluence-to-directional and personal dose equivalent conversion coefficients for neutrons subm. for publ. in Radiat.Prot. Dosim.

[12] H. Schuhmacher, R.A. Hollnagel, B.R.L. Siebert, Sensitivity study of parameters influencing calculations of fluence-to-dose equivalent conversion coefficients for neutrons subm. for publ. in Radiat. Prot. Dosim.

Head of project 2: Dr Thomas

II.1 Objectives for the reporting period

1. Continue measurements of workplace neutron spectra for inclusion in the catalogue of realistic neutron fields.
2. Improve spectrometric instrumentation and data processing techniques in order to provide more reliable spectrometric measurements.
3. Develop and improve the catalogue of realistic neutron fields, incorporating all available spectra and instrument response functions.
4. Utilize the catalogue to investigate dosimetric quantities and dosemeter responses in workplace fields, and to categorise the spectra in terms of their features with a view to providing appropriate calibration facilities.

II.2 Objectives for the next reporting period

1. To analyze the two field measurements performed during the present reporting period.
2. To complete the upgrading of the Bonner sphere (BS) response functions and to integrate the three available spectrometers into a single package.
3. To complete work on developing the catalogue and its manual to make the package suitable for general use.
4. Finalise investigation of dosemeter responses in realistic fields.
5. Investigate the most appropriate calibration fields for realistic workplace spectra with a view to producing them in the laboratory.

III. Progress achieved including publications

III.1 Measurements of neutron spectra

During the year measurements were performed at two locations as part of a series of intercomparison exercises organised by EURADOS Working Group 7.

At the Ringhals nuclear power plant in Sweden a BS set which utilises a gold foil as the central thermal neutron detector was used at a location inside the reactor containment where the dose rates were very high (2mSv h^{-1}) posing severe problems for other spectrometry systems. The induced activities of the gold foils have been measured in preparation for unfolding the spectra.

At the second of the two locations, the NESTOR test reactor at AE Technology, Winfrith, in the UK, three spectrometers were employed, and also a tissue equivalent proportional counter. The three spectrometers were: a BS set with a ^{3}He central detector, an NE 213 scintillator, and a new grided ^{3}He ionization chamber spectrometer. Both the BS system and the NE 213 scintillator performed well, and in fact a preliminary spectrum for the region above 2 MeV was unfolded from the scintillator data during the course of the measurements. Problems with the high thermal sensitivity of the ^{3}He ionization chamber restricted its usefulness, but some very valuable lessons were learnt. The use of the TEPC at Winfrith yielded an independent measurement of neutron dose and dose equivalent, together with gamma dose rate. For the particular configuration employed, about 80% of the dose equivalent was due to photons.

III.2 Improvements to the spectrometry system

Improvements are continuously being made to the spectrometry system with a view to providing a reliable, user friendly, system which combines data from all available spectrometers into a single unfolded spectrum embodying all the available information. Over

the year work has concentrated primarily on the BS unfolding procedure, and the BS response functions. The unfolding procedure has been simplified, particularly in relation to the ease with which different a priori spectra can be tried in the unfolding process.

Recent calculations of BS response functions, performed at NPL, PTB, and GSF with the Monte Carlo transport code MCNP, have indicated that improvements can usefully be made to the response functions which have been used to date for the NPL ^{3}He based system. These response functions were based on measurements in the high energy region (> 1 keV) and at thermal energy, and the use of calculated data from the ANISN code for interpolation. The original ANISN calculations were too high in the thermal region, and although the thermal measurement had been used to adjust the thermal response to the correct average value, the MCNP calculations showed that the shape in the epithermal region above about 0.5 eV was not completely correct. Figure 1 shows the new adjusted response function for the 5" sphere. The spectrum unfolding code STAY'SL was used to perform the adjustment. This is possible because, with STAY'SL, the problem of deriving a best estimate of a response function, based on information from calculations and measurements, is mathematically identical to that of spectrum unfolding. The MCNP code appears well suited to calculating BS response functions although some residual problems connected with detailed modelling of the detectors and the sensitivity to the precise polyethylene density remain to be ironed out.

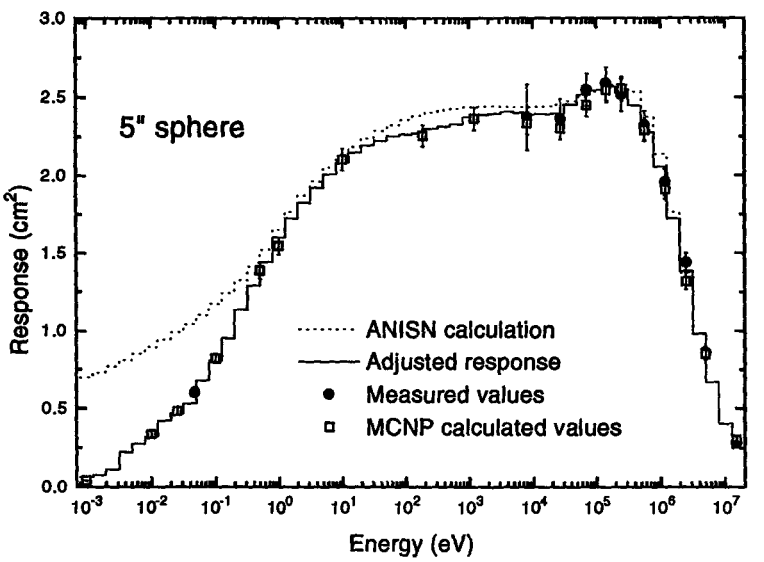


Figure 1. 5" Bonner sphere response function adjustment.

III.3 Improvements and additions to the catalogue of realistic spectra

Work has continued on incorporating new spectral data and new instrument response data into the catalogue. Some of the data were acquired in response to a questionnaire distributed worldwide, others from the literature. The catalogue program has undergone extensive de-bugging, and it has been made simpler to use. A draft manual is available, and it should now be possible to designate a 'frozen' version for trial by 'sympathetic' users.

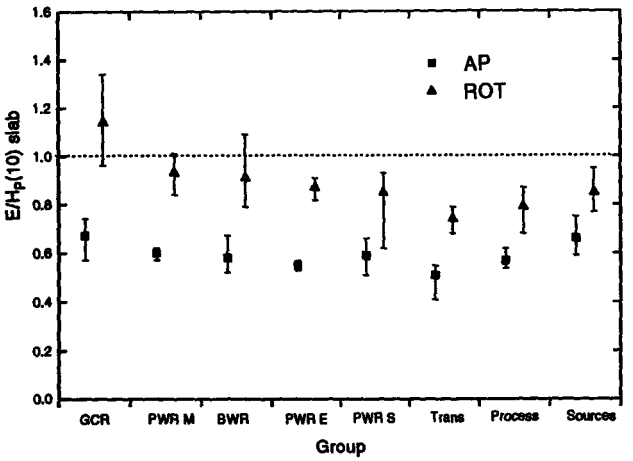


Figure 3. Ratio of effective dose, E, to personal dose equivalent, $H_p(10)$.

neutron fields is open to question. Table 1 quantifies the extent this problem, and shows results for three commonly used area survey instruments for the same groups of realistic spectra described earlier. The table also illustrates how the dosemeter readings depend on the type of field used to calibrate the instrument. The range of under- and over-read which can occur is large, however, the results have significant uncertainties, particularly for the Andersson-Braun and the Studsvik instruments because the data for their responses as a function of energy are far from complete. Work is proceeding to investigate the situation for personal dosemeters where the problem is potentially much more severe.

		HARWELL 0949			ANDERSSON-BRAUN NM2			STUDSVIK		
Spectrum Group (data are mean values for group)	Effective Dose (pSv cm²)	Am-Be	^{252}Cf	D_2O-mod. ^{252}Cf	Am-Be	^{252}Cf	D_2O-mod. ^{252}Cf	Am-Be	^{252}Cf	D_2O-mod. ^{252}Cf
Gas-cooled reactors	20	2.37	2.17	1.66	1.16	1.14	1.07	0.90	0.93	0.89
PWRs in Europe and USA	32	1.80	1.65	1.26	1.04	1.02	0.96	0.84	0.86	0.83
PWRs (Endres and Schraube)	37	1.80	1.65	1.27	1.05	1.03	0.96	0.85	0.88	0.85
PWRs (Endres et al)	39	1.68	1.54	1.18	0.98	0.96	0.90	0.79	0.82	0.79
PWRs (Sanna et al)	51	1.54	1.41	1.08	0.95	0.94	0.88	0.83	0.85	0.82
Fuel transport containers	92	1.21	1.11	0.85	0.81	0.80	0.75	0.74	0.76	0.73
Fuel processing / storage	115	1.21	1.11	0.85	0.95	0.94	0.88	0.81	0.84	0.81
Source environments	187	1.15	1.05	0.81	1.02	1.01	0.94	0.93	0.96	0.92

Table 1. Responses of 3 area survey instruments as a ratio to ambient dose equivalent (calculated with the quality factors of ICRP 60), after calibration in 3 different fields: Am-Be, ^{252}Cf, and D_2O-moderated ^{252}Cf.

III.4 Utilization of the catalogue program

The catalogue is now being used to look at similarities and differences in the measured realistic spectra and to investigate dosemeter responses in these fields. It has also been used to clarify the implications of recent recommendations for changes to dosimetric quantities.

New recommendations for radiation weighting factors for neutrons, which were recently presented in ICRP Publication 60, resulted in serious questions being asked about the continued use of the ICRU operational quantities H*(10), and $H_p(10)$. ICRP 60 proposed the use of a dose limiting quantity, effective dose, E, which is derived from organ doses by applying radiation weighting factors, w_R. Investigation of the ratio of this quantity to the operational quantities as a function of neutron energy revealed large parts of the energy range where the operational quantities for certain irradiation conditions did not have the required property of being conservative overestimates of the effective dose. The significance of this for realistic fields has been investigated.

Figures 2 and 3 show plots of E/H*(10) and $E/H_p(10)$ for eight groups of realistic neutron spectra. The groups, which exhibit increasing values for the effective dose per unit fluence on going from left to right across the figures, are: gas cooled reactors, miscellaneous PWR reactors in Europe and the US, boiling water reactors, fields measured within containment of PWRs by Endres et al. and Sanna et al in the US, transport container environments, nuclear fuel processing areas, and radionuclide source handling areas. Data are shown for AP and PA irradiation conditions, and in Figure 2 also for irradiation which is invariant around a vertical axis through an individual (ROT), and for irradiation from the right and left sides (LAT). The results show that, for realistic neutron fields, the operational quantities are reasonable overestimates of E, however, this happens only because the main contribution to the dosimetric quantities comes from neutrons in the high energy region where the operational quantities do provide an overestimate of E. Potential problems could still arise in any working environment where all the neutrons were of low or intermediate energies.

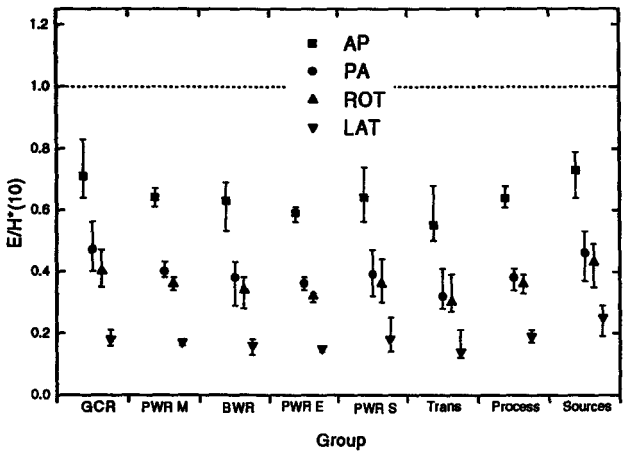


Figure 2. Ratio of effective dose, E, to ambient dose equivalent, H*(10).

Because presently available neutron dosemeters do not have the required dose equivalent response over the full energy range of interest, the reliability of their readings in unknown

Head of project 3: Dr J.L. Chartiert
Scientific staff: Dr. F. Posny, Dr. D. Paul, J. Kurdjian, G. Pelcot, G. Audoin, C. Itié

II. Objectives for the reporting period

1- Improvements of the characterisation of the radiation field produced by the Cadarache 14.6MeV facility ; angular distribution of the neutron spectrum.

2- MCNP calculations for the reference spectra including thermal treatment

3- Realisation of the second facilty: 2.8MeV neutron source. Theoretical study of the monitoring of the target emission.

4- Transfer of a neutron reference to study the response function of proton recoil spectrometers and implement a reference beam line in CEA/BIII. Study of neutron scattering in targets by calculation.

II. Objectives of the next period

1- Development of the 2.8MeV facility in Cadarache laboratory.
Characterisation of the neutron spectra by calculation and measurement.
Intercomparison of dosimetric devices and spectrometers.

2- Bonner sphere technique: response functions of a new ^{3}He "french" counter
Implementation of a well-documented unfolding code.

3- Spectrometry measurements at working places

III Progress achieved including publications

1 - 14.6MeV radiation field

Additional refinements in the simulation of the target assembly have been performed to describe as accurately as possible the interactions in the environment of the beam line and the characteristics (energetic and angular distributions) of the neutron source. The final objective is to evaluate the contributions of the scattered neutrons originating in different parts of the target assembly when an experimental determination cannot be easily performed. Such calculated data have been used to correct fluence measurements in monoenergetic neutron fields produced by accelerators. The same techniques have been also applied in the ultimate version of reference calculated neutron spectra produced at the Cadarache facility.

In the MCNP simulation, the neutron source is represented by a series of "unit sources", emitting in limited solid angles. The juxtaposition of 18 elementary virtual sources enable to describe the angle dependence energy distribution of the target over 4π sr. Another advantage of the composite source is the possibility of accessing to the individual contribution of each unit source. The implementation in the MCNP input file is based, for each one, on an equiprobable energy distribution and an anisotropy coefficient at the mean angle value. A few results have been obtained with the T(d,n)He^4 reaction. They demonstrate that the

"14.6 MeV" contribution in the total neutron spectrum measured on the axis of the beam line (or 0° angle) results, almost exclusively, from neutrons emitted at angle values below 10°. The [10°-20°] and [20°-30°] contributions sum up to less than 2% of the latter, and other can be neglected.

Neutron fluence was investigated at forward direction (0°) and a distance of 50.7cm with the NE213 detector. For the 14.6MeV radiation, a reference measurement has been realized at the BIPM facility (SEVRES) with the same detector. After unfolding the pulse height distribution by the usual technique employed in the laboratory, the 14.6MeV neutron fluence can be derived from the BIPM reference as follows:

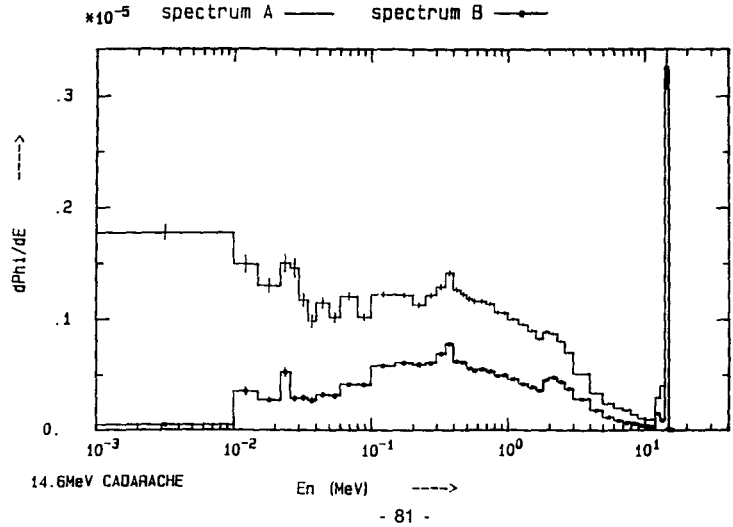
$$\Phi mes(CAD) = \frac{\Sigma n(CAD)}{\Sigma n(BIPM)} . \Phi ref(BIPM) \qquad [1]$$

where Σ n is the number of neutrons with an energy higher than 12MeV. The relation [1] supposes that, above 12MeV, the two response functions are homothetic, hypothese which is true in that case. Then $\Phi mes(CAD)$ = 187.2 cm-2 per monitor count. Because of the mechanical arrangement at the end of the beam line, metallic parts and water cooling system surrounding the emission point, the neutrons emitted in 4π at the target are involved in "parasitic" interactions: scattering processes and attenuation in the target materials. The following figure presents the spectrum calculated at the measurement point (spectrum A) and the spectrum obtained when there is no materials along the neutron path in the forward direction (spectrum B). From these results and the conclusions deduced from the calculations described before, it can be stated that:

- the events recorded above 12MeV are mainly due to neutrons directly emitted by the target. Only 2% can be considered as scattered neutron and have to be substracted from the fluence value determined by the relation [1].

- the proportion of neutrons which have interacted with the target materials in the forward direction is determined by the ratio of the fluences spectrum A/spectrum B in the energy region above 12MeV. It has been found to be 4.5%.

Consequently, the 14.6MeV fluence at the measurement point can be considered to be 191.7 cm-2 per monitor count.



All these efforts to estimate this fluence have been realized to have an indirect mean to check the calibration of the monitoring system. The relation between the number of monitor counts and the number of neutron emitted in 4π at the target involves the knowledge of the measurement solid angle of the diodes used as detectors in the monitoring system. For now, the solid angle value was determined with the mechanical data of the setup and found to be equal to 2.49 10^{-6} sr. The previous measurement leads to a value of 2.48 10^{-6} sr. This good agreement allows to confirm the conversion factor of $5.94.10^{6}$ monitor counts for 1 neutron emitted in 4π at the target.

2. MCNP calculations of reference neutron spectra

By applying the "thermal treatment" $S(\alpha,\beta)$ option to hydrogen in polyethylene, MCNP calculations have been performed for the two configurations (denoted CI and CII) of the 14.6MeV Cadarache facility, involved in irradiations of dosimetric devices and the spectrometry intercomparison of 1991. In CII, an addittional water filter (thickness : 20cm) modifies mainly the high energy part of the neutron spectrum. Although experiments have been performed by using CII, calculated data have not yet been validated by spectrometry. A comparison with previous data obtained without thermal treatment shows an increase of total fluence lower than 3%.

Configuration I - [14.6 MeV+238U+Fe] in PE duct

Neutron fluence(*)			Dose equivalent(*)			
Total	[13-15]MeV	[<1eV]		Total	[13-15]MeV	[<1eV]
3.15(-5)	0.4%	34%	ICRP21	2.03(-13)	2.5%	6.0%
			ICRU39	2.34(-13)	2.8%	4.2%

Configuration II - [14.6 MeV+238U+Fe+H2O] in PE duct

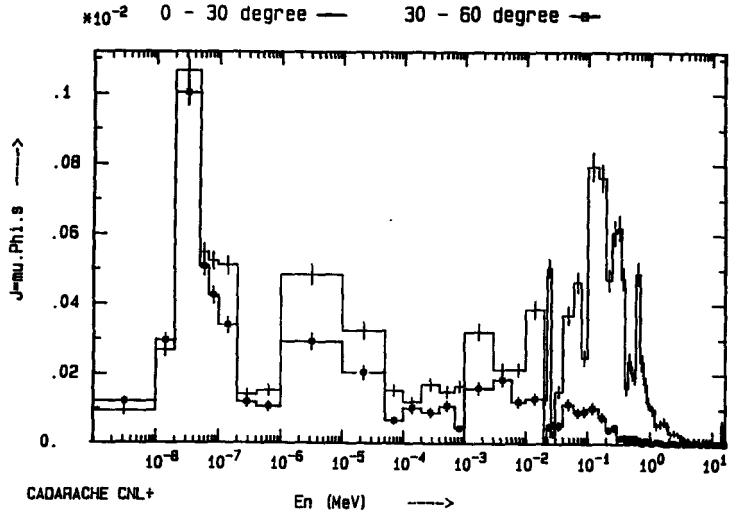
Neutron fluence(*)			Dose equivalent(*)			
Total	[13-15MeV]	[<1eV]		Total	[13-15MeV]	[<1eV]
1.70(-5)	0.1%	55%	ICRP21	4.9(-14)	1.8%	21%
			ICRU39	5.2(-14)	2.3%	

(*) for 1 neutron in 4π sr.

Angular distribution of the neutron spectrum

Several characteristics of the neutron field produced by the Cadarache facility have been thoroughly studied for the last years. But the objective of using that facility to calibrate dosimetric devices implies to have, in addition, a knowledge of the angular distribution of the neutrons. The cross-section of the beam, at the exit of the polyethylene duct, is rather large (70cm in diameter). Therefore, the calibration zone is irradiated by neutrons having an extended range of incidence angles. This question has to be taken into account, in particular when the angular response of an instrument is involved, or has to be studied.

The MCNP code has been run to investigate the angular distribution of neutrons in a plane perpendicular to the symetry axis, at the calibration distance (30 cm from the exit plane). The following graphs represent the energy distribution of the neutron current respectively in the angular ranges (0°->30°)and (30°->60°). They show clearly differences in the high energy part of the spectrum above 10 keV, confirming some anisotropy in the neutron field. A study is in progress to correct this effect.



Preliminary results have shown the interest of using as a primary neutron source the D(d,n)He^3 reaction yielding a "2.8 MeV" neutron field. A facility based on a SAMES T4OO accelerator (max.H.V. 400kV), is being realized. In addition to the technical problems related to engineering (mechanics,vacuum techniques), a specific study has been undertaken to evaluate the characteristics of the monitoring system based on the competitive reaction D(d,p)T. Proton counting is deemed a satisfactory solution, with respect to the detection of charged particles to monitor the neutron emission of the target.

Due to the fact that two nuclear reactions are involved in the "(d,d)" process and that both reactions are markedly anisotropic in the C.M. system, a more complex situation has to be dealt with to calculate the neutron yield per proton measured in a solid angle.The quantities of interest are respectively Yn and yn :

Yn = number of neutrons in 4π sr per proton in $\Delta\Omega p$ at 176°

yn = number of neutrons in 1 sr at O° per proton at 176°

In practice, real targets are considered as "thick" targets. The determination of Yn and yn has been made for an assembly of N "thin" targets to approximate the transport of the incident deuterons through the target. They are given by the relations hereafter :

$$Yn=(1/N).(4\pi/\Delta\Omega p).\Sigma Kp(Ed).R(Ed)$$

$$yn=(1/N).(1/\Delta\Omega p).\Sigma(Kp(Ed)/Kn(Ed)).R(Ed)$$

taking into account the kinematics. the cross-sections and the energies involved in the reactions. For operational conditions of the SAMES T.400 (Ed=320keV, target thickness=500$\mu g/cm^2$, $\Delta\Omega p$=2.49 10^{-06} sr), the following results have been obtained.

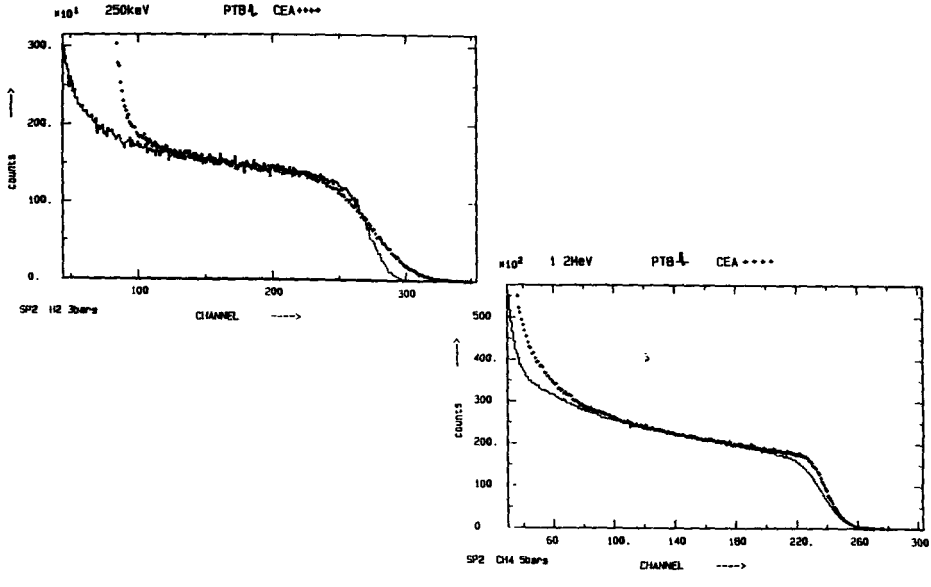
$Yn=0.478\ 10^7$ n in 4π sr/(p at 176°)

$yn=0.726\ 10^6$ n in 1 sr at 0°/(p at 176°)

4. Neutron reference fluence and SP2 response functions

An accurate knowledge of the response functions is essential to achieve a good estimation of the neutron spectrum. This concerns the shape, the energy calibration, the efficiency and the energetic resolution at several energies. Because the monoenergetic references in France are not enough reliable, a joined study has been undertaken with PTB leading to the transfer of the PTB references to the CEA facility. A first set of measurements with the SP2 counters have been performed at PTB in december 1992 at six energies between 144keV and 1.2MeV and compared to previous measurements realized at CEA.

Besides these measurements, calculations with the MCNP code have been started to estimate the contribution of the neutrons scattered in the target holder of both facilities. Two examples are presented on the following figures on which the response functions have been normalized at the mid-neutron energy.



These preliminary results allow two important conclusions:

- the shapes are similar in the linear part of the response function. From the value of this slope is deduced the correction factor applied to the shape calculated by the Snidow formulation.
- the channel numbers corresponding to the neutron energies are also in good agreement, confirming the previous energy calibration.

The efficiency values presented in the following table have been corrected for the contribution of the neutrons scattered in the target holder (except at 875keV at which no calculation is available for now), making the hypothese that all these neutrons have been recorded by the detector: 3.6, 5.8, 1.8 and 4% of the total fluence at respectively 144, 250, 565 and 1200keV.
As some values are greater then 100%, the data involved in the efficiency determination have to be checked, i.e. the exact inner volume of the counter, the number of hydrogen atoms and the (n,p) cross-sections.

SP2 counter	H2 3bars			CH4 5bars		
Eneutron (keV)	144	250	565	565	875	1200
Eneutron position (channel)	150	262	584	111	175	236
Efficiency (%)	94	94	107	103	104*	98
Resolution (FWHM %)	13	11	10	10	9	12

At the CEA facility a new line beam has been devoted to the fluence transfer and new measurements have to be performed before any attempt of conclusion.

Head of Project 4: Dr.H.Schraube
Scientific technical staff: Dr.J.Jakes, Dr.G.Leuthold, V.Mares, G.Schraube, E.Weitzenegger

II. Objectives for the reporting period

1. Calculation of the response matrix to a BS system with ^{6}LiI detector
2. Calculation of organ doses and derivation of a modified radiation weighting factor

III. Progress achieved including publications

Ad 1. Bonner sphere responses

The unfolding and evaluation of the spectral neutron fluences collected or experimentally determined in the frame of this contract suffered from the imperfect knowledge of the response matrix of the Bonner sphere system with LiI(Eu) detector. Although a number of publications was dedicated to this problem in the past, the results were not in agreement with recent experimental findings. The reason is that most of them applied the multigroup transport code ANISN, which could not account for the chemical binding of the hydrogen in the polyethylene material of the spheres, could not model sufficiently precisely the thermal neutron detector assembly, and permitted only a course energy binning. Therefore, the total matrix was calculated for a LiI cristal of 4mm diam. x 4mm height applying the Monte-Carlo code MCNP version 4. One result is shown in figure 1: For each of the available 86 experimental energy points (Alevra et al.,1992, Thomas et al., 1993) the calculated response was divided by the experimental one. This resulted in a mean calibration factor of 0.72, with 9% standard deviation and 1% standard error of the mean. The calibration factor descibes quantitatively the light event loss in the optical system.

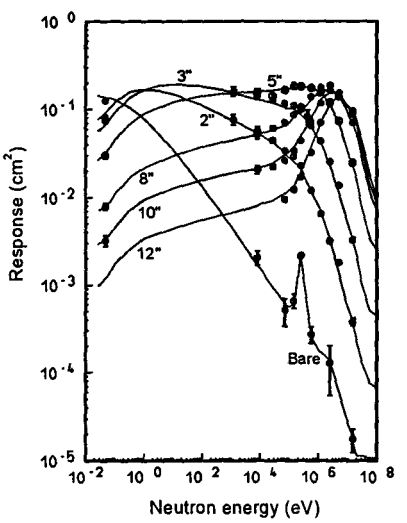


Figure 1: MCNP calculated and experimentally determined responses of Bonner spheres with 4x4mm ^{6}LiI(Eu) detector. The error bars at the experimental data points depict the total (1σ) uncertainties, the lines connect the calculated data points.

Statistical checks indicated that no systematic deviations between calculated and experimental data were present, in contrast to comparisons with earlier calculations. Furthermore, it was found that a hybrid log-normal distribution versus the sphere mass (instead of the sphere diameter) gives an excellent possibility of interpolating the responses in the sphere size domain.

Ad 2. Organ doses and radiation weighting factor
Our calculations of the organ doses in antropomorphic phantoms led to the conclusion that the ICRP proposals on the introduction of the radiation weighting factor results in an

considerable overestimation of the body doses, compared with the ICRP51/ICRU43 concept. Therefore, modifications were considered and determined, by altering the numerical values of the radiation weighting factor, but retaining the concept. The first modification (see figure 2 and 3) was choosen as a conservative envelope of all organ related quality factors, ie the ratio of dose equivalent to energy dose of the respective organ. The second one was done by defining the radiation weighting factor as the ratio of the whole body dose equivalent to the whole body dose for AP radiation incidence. Both modifications can, of course, not eliminate the assumption of ICRP60 that the high and low LET components inside the phantom do not change. By the modifications, the overestimation is reduced and, as a accompanying effect, the ICRU defined quantity ambient dose equivalent remains conservative up to 40MeV.

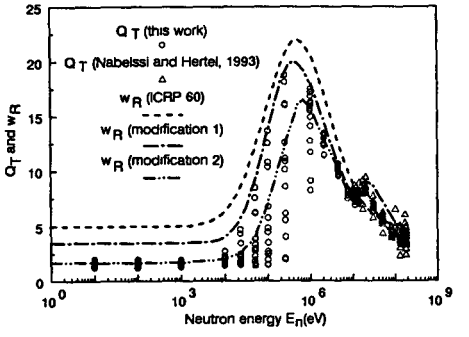


Figure 2

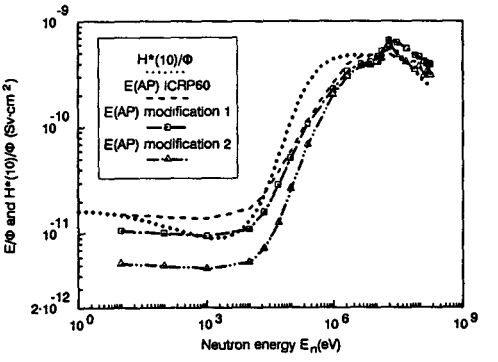


Figure 3

Figure 2: Quality factors Q_T of seven important organs compared with the radiation weighting factor w_R and the two proposed modifications (each symbol depicts a certain organ and a certain irradiation incidence to the body. Here, the symbols are not referenced in order to keep clearness)

Figure 3: Fluence-to-effective dose equivalent conversion function applying the ICRP radiation weighting factor and the two modifications.

References

A.V.Alevra, M.Cosack, J.B.Hunt, D.J.Thomas,and H.Schraube: Experimental determination of the response of four Bonner sphere sets to monoenergetic neutrons (II). Radiat.Prot.Dosim. 40,2 (1992) 91-102

V.Mares and H.Schraube (1993), G.Leuthold and H.Schraube (1993), D.J.Thomas et al. (1993) see Publications

Publications

V.Mares and H.Schraube: Evaluation of the response matrix of a Bonner sphere spectrometer with ^{6}LiI detector from thermal energy to 100MeV. Accepted for publication in Nucl.Instr.Meth.Radiat.Res. A (1993)

G.Leuthold and H.Schraube: Critical analysis of the ICRP60 proposals for neutron radiation and a possible solution. Presented at the Villigen-Workshop (1993). Submitted to Radiat.Prot.Dosim.

D.J.Thomas, A.V.Alevra, J.B.Hunt, and H.Schraube: Experimental determination of the response of four Bonner sphere sets to thermal neutrons. submitted to Radiat.Prot.Dosim. (1993)

Progress Report

Contract: FI3P-CT920018 Sector: A12

Title: The measurement of environmental radiation doses and dose rates.

1)	Bøtter-Jensen	Lab. Risø
2)	Lauterbach	PTB
3)	Delgado Martínez	CIEMAT
4)	Pernicka	IRD

I. Summary of Project Global Objectives and Achievements

In order to monitor changes in the level of ambient photon radiation, instruments and dosemeters are required which have both adequate sensitivity and sufficient accuracy to record small variations. To ensure that such measuring devices, which are typically ionisation chambers, scintillators, GM counters and integrating electronic and TL dosemeters, give reliable measurement results, it is necessary to determine the response of the detectors to both cosmic radiation and to a terrestrial spectrum.

Based on the effort put on the evaluation of practical calibration methods for environmental radiation monitoring systems, including integrating TL dosemeters, and the determinations of a variety of detector characteristics carried out as a successful collaboration between Risø, PTB and CIEMAT in 1991/92, this work was continued by studying further important aspects of environmental radiation measurements.

A Natural Environmental Monitoring Station was established at Risø with the aim of being able to offer EC member states reference measurements at a site with a well determined natural terrestrial photon spectrum. Long-term measurements using different detectors will be made and special emphasis put on measuring and analysing the contribution to the detector responses from radon daughters. It is considered important to initiate long term studies of detector responses to radon daughters as a basis for the evaluation and recommendation of suitable alarm criteria for national radiation monitoring networks. Contacts were established with authorities in the USA with the purpose of initiating US-European collaborative work on methods connected with environmental radiation measurements.

New types of electronic dosemeters and TL dosemeter materials were tested at the new terrestrial measurement station at Risø as well as in the UDO underground laboratory in the Asse saltmine (PTB). Experiments in Asse were set up to study the sensitivity, linearity and inherent background of new types of dose rate meters and electronic dosemeters. Monte Carlo calculations were initiated at Risø in collaboration with PTB to determine the scattered radiation from a collimated beam calibration set up in the Asse mine. Experiments carried out by CIEMAT in the UDO laboratory were concerned with the investigation of self-dose contributions to the response of some sensitive TL materials. The newly introduced highly sensitive LiF:Mg,Cu:P TL material was further investigated at CIEMAT and experience was gained using a new glow curve analysis technique for measuring environmental doses over ultra short integration times (1 day).

Within the reporting period the Institute of Radiation Dosimetry (IRD) in Prague became a new associated partner of the project. IRD has already participated in project coordinating meetings and in intercalibration experiments carried out at Risø.

Head of project 1: Dr. Bøtter-Jensen

II. Objectives for the reporting period

a) Commissioning of established free-field and shadow-shield calibration facilities.
b) Evaluation of measurements at the Hinkley Point Nuclear Power Station.
c) Establishment of an Environmental Terrestrial Measurement Station at Risø.
d) Development of software for assessing dose rates and contributions from radon daughters.
e) Initial long-term measurements at the Risø Environmental Station.
f) Monte Carlo calculations of scattered radiation in the Asse laboratory.
g) Contacts with US authorities to form an official collaboration on environmental radiation measurements.
h) Implementation of glow curve analysis techniques into the Risø TL environmental monitoring procedures.

Objectives for the next period

- Further documentation of the above points.
- Long-term measurements at the Risø Terrestrial Measurement Station.
- Establishment of a Cosmic Measurement Station at Risø.
- Incorporation of new partners from Eastern Europe.
- Comparison of TLD, electronic dosemeters and active dose rate meters.
- US-European intercalibration at Risø
- Preparation of publications and final EC report.

III. Progress achieved including publications

a) The free-field and shadow-shield calibration facilities established at Risø during a previous phase of the EC project were commissioned by determinig the scattered gamma photon contributions to the detector responses in different calibration geometries. Monte Carlo calculations based on the MCNP code were carried out for a variety of source-detector geometries using certificated Co-60, Cs-137 and Ra-226 gamma sources, and the results were analysed and published (Bøtter-Jensen and Hedemann Jensen, 1992). It was concluded from both calculations and experiments that the scattered radiation from surrounding buildings farther away than 15 m from a free-field set-up contributes negligibly to the detector response relative to that from ground and air. It was further shown that the dose rate measured by a detector at a source-to-detector distance of 5 metres in a shadow-shield set-up typically has a scattered radiation component of about 60%.

b) Measurement data obtained from experiments carried out at the Hinkley Point Nuclear Power Station in the UK with the aim of comparing the responses from three different types of dose rate meters and a variety of TL materials were recently evaluated and analysed (I.M.G. Thompson et al, 1993). An attempt was made to determine the separate dose rate contributions from the different

ambient radiation components present at this special location including that from the periodic release of Ar-41.

c) An Environmental Terrestrial Measurement Station was established at a field site at Risø with the aim of being able to offer reference measurements at a well determined natural environmental photon spectrum. 6000 m^2 (60x100 m) of agricultural land inside the fence of the Risø laboratories were adopted - kindly offered by the Risø management - especially for this particular purpose. The new test site is situated far from laboratory buildings, and the detectors to be tested there will be unaffected by the nuclear installations at Risø. It was considered important that the station could be established within the controlled area at Risø as a precaution against vandalism during long-term testing of expensive monitoring equipment. An instrument control hut made of wood was designed and built at the field site which contains data loggers and controlling computers. Power lines, telephone and a data net were installed which will permit the on-line transfer of data to any place in the world. Precipitation, air pressure and temperature gauges were also installed which are continously monitored by a computer. A schematic diagram of the new measurement station and details of the instrument control hut are shown in Figures 1A and 1B.

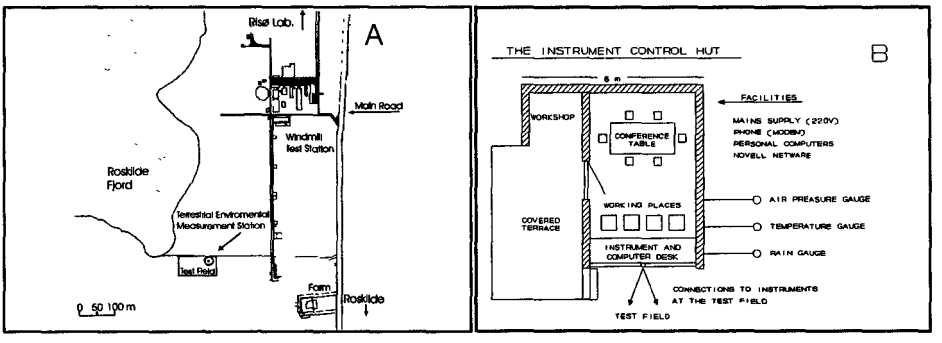
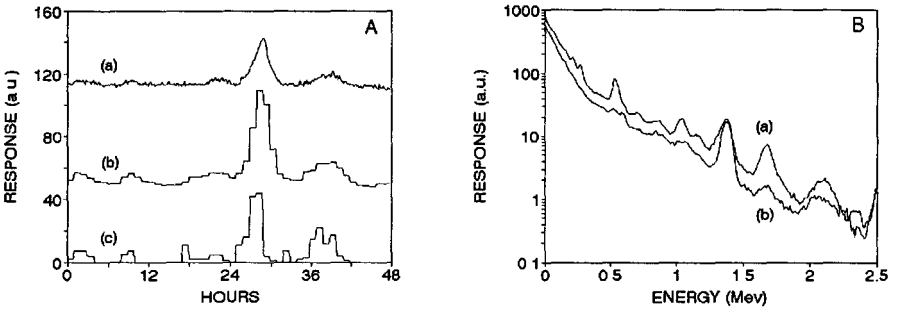


Fig. 1. Schematic diagrams of A) the Risø Environmental Terrestrial Measurement Station and B) the instrument control hut.

d) Computer software was developed to allow one PC to control a number of environmental dose rate meters over a prolonged time. The computer screen displays real time dose rates measured by the different detectors as a function of time together with precipitation, air pressure and temperature data, and the results are automatically stored so that any event can be recalled and analysed. The software also provides the display of real time gamma spectra obtained by a 3x3" NaI detector connected to a multi-channel analyser, and the spectra are continuously stored. Special emphasis was placed on the evaluation of the contribution to the ambient radiation from radon daughters as a result of radon escape from the soil. These disturbing "radon peaks" play an important role in assessing action levels in connection with national early warning systems installed in many countries. For this particular task a special curve fitting software was

developed and incorporated with the aim of being able to distinguish between K-40 and Rn-daughter gamma peaks by comparing with two standard spectra. The result is a response curve representing the radon daughter concentration only as a function of time. Studies of the statistical radon daughter events and their impact on measuring the environmental radiation background have been initiated and data are being collected together with precipitation, air pressure and temperature data for further detailed analysis. Fig. 2A shows typical responses to the natural environmental radiation obtained over 24 hours from a high pressure ionisation chamber and the NaI spectrometer. A typical "radon peak" measured with the ionisation chamber is clearly demonstrated. Fig. 2B shows typical gamma spectra obtained from the same period.

Fig. 2. A: Typical measurement results obtained over 24 hours at



the Risø Environmental Station from (a) the high pressure ionisation chamber, (b) the NaI spectrometer (radon daughter window) and (c) the precipitation gauge. The "radon peak" associated with rain fall is clearly seen. B: Typical gamma spectra obtained from the same period. Spectrum (a) is affected by a high radon concentration and (b) is the "normal" environmental spectrum.

e) Initial measurements at the new Risø Environmental Measurement Station were carried out in connection with a project meeting in May 1993. Free field calibration experiments using Cs-137, Co-60 and Ra-226 certificated sources were performed and a variety of new types of environmental dose rate meters and electronic dosemeters were tested. Long-term measurements were initiated using a Reuter Stokes high pressure ionisation chamber, a GEC GM environmental monitor, an Alnor GM environmental monitoring system and a 3x3" NaI Scintillator. A typical dose rate response to the natural background (terrestrial plus cosmic radiation) for the high pressure ionisation chamber as a function of time is shown in Fig.3A, and the responses to the ambient radiation for a variety of electronic dosemeters over the same time period are shown in Fig.3B. The new electronic dosemeter developed at NRPB and provided by Plessey was tested and compared with a GEC GM environmental monitor over a three month integration period in a natural radiation environment in the UK. Preliminary results

showed that the GEC GM environmental monitor read an integrated air kerma of 112.4 μGy compared with the Plessey electronic dosemeters which read a penetrating dose equivalent of 136 μSv and a skin dose equivalent of 144 μSv. The conversion of these quantities is complex and is being investigated.

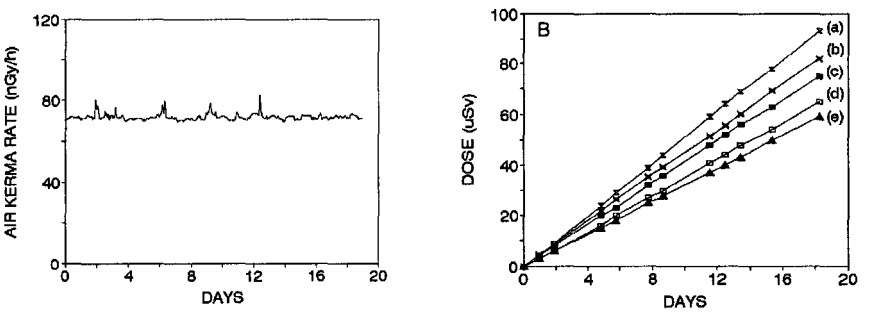


Fig. 3. A: Typical dose rate response to the natural environmental radiation (terrestrial plus cosmic) over 20 days for the high pressure ionisation chamber. The peaks caused by radon daughters can be clearly seen. B: Responses to the environmental radiation from a variety of electronic dosemeters over the same period (a) Gothic Crellon (Bleeper 1542), (b) SAIC (114), (c) Alnor (RAD-101), (d) Stephen (GAMMACOM 4200) and (e) Appleford Instr. (AI-1/STD).

f) Risø has initiated Monte Carlo calculations to determine the scattered gamma photons from a collimated beam calibration set-up in the Asse underground laboratory that uses low-active Cs-137 gamma sources. PTB provided detailed drawings of the facility and on this basis Risø constructed a geometry model for the MCNP code. Initial calculations have been made to evaluate the scattered contribution to the direct collimated beam from interactions in the collimator construction and the walls of the cave using different Cs-137 sources previously used for intercalibration experiments.

g) Contacts were established with different institutions in the USA dealing with environmental radiation measurements aiming at a collaborative work on standardising calibration methods etc. The reactions have generally been very positive, and it is anticipated that experts from the USA would be able to participate in US-European intercalibration experiments planned to be carried out at Risø in the spring of 1994. Fundings for US experts to travel to Europe seems to be the main problem. It is considered very important that a link is formed between laboratories in the USA and Europe to enable a common effort to be put on the standardisation of methods for environmental radiation measurements.

h) The implementation of the glow curve analysis technique developed at CIEMAT into the Risø TL environmental monitoring routines was initiated as a collaboration between Risø and CIEMAT by testing a programme especially designed for non-linear heating of TL dosemeters as is the case with the N2-heating used for routines at Risø. The obtained results have been analysed and published (Gomez Ros et al, 1993).

Publications

- Determination of Scattered Gamma Radiation in the Calibration of Environmental Dose Rate Meters. L.Bøtter-Jensen and P.Hedemann Jensen. Radiat. Prot. Dosim. **42** 291-299 (1992)

- The Assessment of External Photon Dose Rate in the Vicinity of Nuclear Power Stations; An Intercomparison of Different Monitoring Systems. I.M.G.Thompson, L.Bøtter-Jensen, U.Lauterbach, W.Pessara, J.C.Saez-Vergara and A.Delgado. Accepted for publication in Radiat. Prot. Dosim. (1993).

- A Glow Curve Analysis Method for Non-Linear Heating Hot Gas readers. J.M.Gomez Ros, J.L.Muniz, A.Delgado, L.Bøtter-Jensen and F.Jørgensen. Radiat. Prot. Dosim. **47** 483-488 (1993).

- Comparison of Main Thermoluminescent Properties of some TL Dosemeters. M.Prokic and L.Bøtter-Jensen. Radiat. Prot Dosim. **47** 195-199 (1993).

Head of project 2: Dr. Lauterbach

II. Objectives for the reporting period

1. Investigation of the inherent background of electronic dosemeters

2. In cooperation with the Ciemat continuation of the work in the field of TL-dosemeters
   - self-dose contribution

3. In cooperation with the Risø National Laboratory Monte Carlo calculations of the radiation fields of the irradiation facility in the UDO laboratory
   - collecting all data for the Monte Carlo calculations

4. Publication

III. Progress achieved including publications:

1. Investigation of the inherent background of electronic dosemeters

The knowledge of the composition and the dose rate of the background radiation level in the UDO laboratory is essential for the investigation of the inherent background of electronic dosemeters.

First measurements of the gamma ray spectra in the laboratory showed that the predominant sources of the background radiation are ^{40}K in the surrounding rock salt, natural radionuclides of the Uranium-Radium-series and a low contamination of the wooden framework below the laboratory building with ^{137}Cs and ^{134}Cs. But the contamination of the framework is neglegible.
As a follow up of the earlier research work long term measurements of the dose rate in the Asse laboratory UDO were performed combined with dose rate measurements inside the special 5 cm thick lead shield in the laboratory.
The reading R_{TOTAL1} of the used dose rate meter in the laboratory is given by the following equation (1):

$$R_{TOTAL1} = \{r_K(\dot{K}_K) + r_{Ra}(\dot{K}_{Ra})\} + R_i \qquad (1)$$

where: r_K, r_{Ra}, are the responses of the detector to ^{40}K-radiation and to the radiation from radionuclides of the Uranium-Radium-series respectively,
$\dot{K}_K$ and $\dot{K}_{Ra}$ are the "true" air kerma rates from the mentioned radionuclides and R_i is the contribution to the reading of the instrument from internal radioactive contamination and/or non-radiation reading, such as leakage current.
For the reading inside the lead shield the attenuation of each component of the background radiation has to be taken into account. In equation (2) F_K and F_{Ra} are the dose attenuation rates for the considered radiations. The reading R_{TOTAL2} is given by

$$R_{TOTAL2} = \{(1/F_K)r_K(\dot{K}_K) + (1/F_{Ra})\, r_{Ra}(\dot{K}_{Ra})\} + R_i \qquad (2)$$

Equations (1) and (2) can be rearranged on the assumption that the contribution of both radiation sources to the total air kerma rate of the background radiation is in the order of 50 %, the dose attenuation rates and responses of the detector are known. Solving this equation system the total background air kerma rates $\dot{K}_B$ in the laboratory UDO and inside the

lead shield can be calculated according to equation (3)

$$\dot{K}_B = 0.5\,\dot{K}_K + 0.5\,\dot{K}_{Ra} \qquad (3).$$

The solution of this equation sytem yields the inherent background R_i of the detector, too. The equivalent air kerma rate of R_i is 12.39 ±0.15 nGy/h.

Table 1 shows the responses of the used detectoer FHZ 600 A and the dose attenuation rates

Table 1

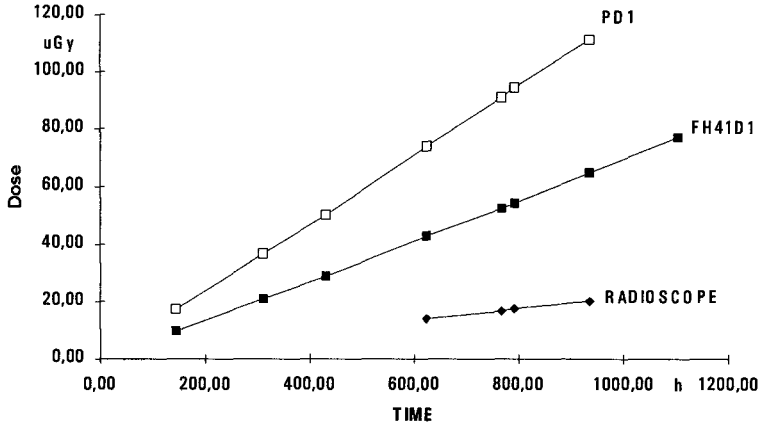
	^{40}K	Uranium-Radium-series
Response	1.42	1.12
Dose attenuation rates	12.5	16.7

The readings R_{TOTAL1} and R_{TOTAL2} and the calculated total air kerma rates $\dot{K}_B$ are presented in Table 2.

Table 2

	R_{TOTAL} nGy/h	K_B nGy/h
UDO	13.27 ± 0.15	0.69 ± 0.15
Lead shield	12.45 ± 0.15	0.061 ± 0.12

The inherent background of three types of electronic dosemeters have been investigated in the UDO ultra low background facility in the Asse salt mine. The dosemeters are battery operated and have a Geiger-Müller counter as detector. The instruments are designed as pocket dosemeters and they are able to indicate the accumulated dose.



Readings of the accumulated doses of the electronic dosemeters

The diagram shows the dose readings in μGy during a period of several weeks. The measurements demonstrate considerable differences of the indicated inherent background. The slopes

of the straigth lines yield equivalent air kerma rates for the inherent background which vary between 20 nGy/h and 120 nGy/h. Table 3 shows these values R_{TOTAL1} including the real background in the UDO facility in nGy/h which were derived from the slopes. The estimated real background in UDO is 0.69 nGy/h and does not affect significantly the indicated accumulated doses of the three instruments.

Table 3

Manufacturer	SAIC	FAG Kugelfischer	Münchener Apparatebau
Instrument	PD-1	FH41D1	RADIOSCOPE
R_{TOTAL1} nGy/h	119.04	70.21	19.74

The relative high inherent background is supposed to be caused mainly by the inherent radioactivity of the instruments. Preliminary measurements of the dosemeters with a high purity germanium spectrometer showed that the 1461 keV γ-line of ^{40}K increased by a factor of two compared with the environmental spectrum in UDO. Removing the battery from the instrument reduced the pulse rate in the 1461 keV γ-line to the environmental level.
It is supposed that the inherent background is mainly due to the ^{40}K in the battery. More detailed spectrometric measurements and experiments with a remote power supply for the dosemeters will be performed to investigate the source of the built-in radioactivity.
The constant slope during the whole measuring period exhibits a considerable linear dose accumulation with time at the very low dose rates due to the inherent radioactivity.

2. In cooperation with the Ciemat continuation of the work in the field of TL-dosemeters

The knowledge of the composition and the dose rate of the background radiation level in the UDO laboratory is essential for the determination of the self-dose contribution of TL-dosemeters. The estimated value of the environmental air kerma rate in UDO (see section 1) is also the basis for the evaluation of the self-dose experiments.

3. In cooperation with the Risø National Laboratory Monte Carlo calculations for the irradiation facility in UDO

A set of data concerning the geometry of the collimated beam arrangement, the design of the sources, the dimensions and the construction materials of the laboratory building was submitted to the Risø National Laboratory as the basis for the Monte Carlo calculations. The results of these calculations are to be compared with the existing air kerma rate measurements in UDO.

4. Publication

W. Peßara, U. Lauterbach: An extreme low-level underground laboratory for dosimetry and spectrometry (UDO), to be published in: Umweltradioaktivität Radioökologie Strahlenwirkungen, Tagung des Fachverbandes Strahlenschutz, Binz auf Rügen 1993.

Head of Project 3: Dr. Delgado Martinez

II.- Objectives of the reporting period

a) Study of the low dose (μGy) performance of LiF:Mg,Cu;P using the new CIEMAT computerized evaluation method. Application to ultrashort integration (1 day) environmental dosimetry.
b) Completion of the Asse experiment on selfdosing after 6 months exposure inside the UDO laboratory.
c) Characterization of the TLD-100 peak 4 thermal behaviour with reference to the important glow curve changes detected in real environmental conditions.
d) Comparison of TLD-100/ Reuter Stokes dose results in long term ambient exposures (1 to 6 months).
e) Participation in common activities together with Risø and PTB for the intercomparison of different types of dosemeters.

III.- Objectives for the next period

a) Refinement of the computer programme for LiF:Mg,Cu,P after the experience gained with the first version.(CIEMAT).
b) Check of the very short term environmental dose measurement capability of LiF:Mg,Cu,P (RISØ monitoring field station).
c) Linearity of LiF detectors at very low dose rates. Dose rate effects. (Irradiations with the UDO certified sources).
d) Study of TLDs response at high energy photons.(CIEMAT-PTB).
e) Completion of the TLD-100/Reuter Stokes comparison and determination of the TLD-100 response to cosmic radiations under different plastic thicknesses.(CIEMAT-RISØ station).

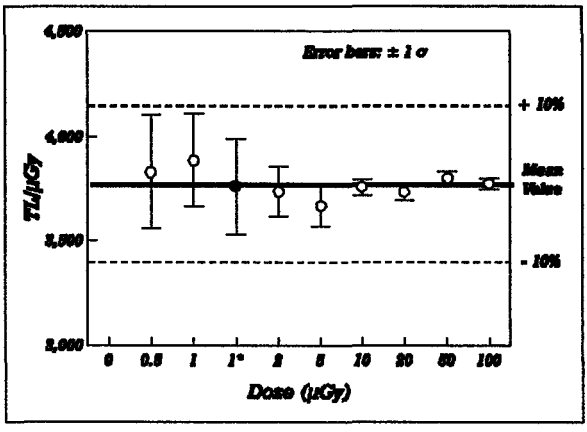
IV.- Progress achieved including publications

a) Intensive work has been devoted to the study of the features of the response of LiF:Mg,Cu;P (GR-200) in the dose range 0.5-100 μGy. It is known that the maximum temperature allowed to preserve the material properties is insufficiently low to anneal completely some radiation induced high temperature peaks, thus determining the presence of unavoidable dose dependent residual signals. This fact limits the usefulness of this high sensitivity, tissue equivalent material.

After a careful study of these unwanted residual signals we have succeeded in establishing relatively simple criteria separating these residuals from the dosimetric peaks, lying at lower temperatures. These criteria can be implemented in a computer programme performing the dosimetric evaluation of the LiF GR-200 glow curves. This programme treats the residual high temperature components as a kind of background, variable in intensity depending on previous doses. With this programme reliable and accurate dose measurement performance can be achieved in the range 0.5-100 μGy using only reader anneal (linear up to 255 °C). Using oven anneal (10 minutes at 240°C) this range can be extended. These results open the possibility of measuring environmental doses integrating over very short periods, even shorter than one day, as has been demonstrated by recent results obtained at CIEMAT. A seminar "Measurements of very low doses

(μGy) with LiF:Mg,Cu,P" was given at RISØ National Laboratory on May 1993, presenting the results obtained so far.

Figure 1 presents the mean values (TL/μGy), obtained from a group of five detectors, for ten consecutive irradiations at the indicated absorbed dose values in the range 0.5-100 μGy. Error bars correspond to one standard deviation (one measurement). At 1 μGy two points are represented. The open point is the mean value obtained from the ten consecutive irradiation at that dose. The closed point corresponds to the mean value of another group of tests at 1μGy. Each test is performed after each one of the series at the different dose values. The closeness of both the mean value and the standard deviation of the two groups of 1 μGy irradiations can be appreciated. This good agreement assures that 1 μGy can be properly measured no matter the doses previously received within the range studied.



b) Several materials currently employed for environmental monitoring were tested for selfdosing effects. LiF TLD-100 and GR-200, CaF_2:Mn, CaF_2:Dy, SO_4Ca:Dy and some types of Panasonic badges were studied. The experiment included storage for six months of samples of these materials in the ultralow ambient dose UDO laboratory, plus controlled irradiations for calibration purposes, also performed inside the Asse mine, using certified gamma sources. The experiment was completed very recently (August 1993) and the results are still under study.

It can be advanced that the data from the two LiF varieties, TLD-100 and GR-200, are particularly interesting. They reveal the absence of any detectable selfdose contribution. From TLD-100 samples only background signals are detected, distinguishable from true radiation induced TL peaks by the use of glow curve analysis. In the case of GR-200, much more sensitive than TLD-100, a measurable TL signal is detected. Using the GR-200 glow curve analysis programme and a provisional dose calibration (a

more refined one will be received from PTB soon) the deduced environmental dose rate is lower than 1 nGy/h, in good agreement with the reported value inside the UDO laboratory.

c) In previous studies, already published, on temperature effects during long ambient exposures, we identified peak 4 as the major cause of the TLD-100 complex response when stored at ambient temperatures. The intriguing peak 4 behaviour led us to develop a laboratory experiment to try to get more information on the peak 4 characteristics. TLD-100 glow curves were measured after different cooling rates and readout conditions. From very fast coolings: quenching into acetone to rather slow ones:1ºC/s. Readouts between 16ºC/s and 1ºC/s were employed. Very small and thin samples were employed to minimize thermal lags across the samples and keep glow curve distortions to a minimum.

From the results obtained it can be concluded that peak 4 is the origin of most of the TLD-100 practical difficulties. This peak is extremely dependent on the characteristics of every thermal step or treatment involved in the use of TLD-100 dosemeters, as for example exposure at ambient temperatures. From a theoretical point of view the peak 4 behaviour can not be explained in terms of the usual Randall-Wilkins scheme for TL processes and one has to search for more complex models involving trap modification processes during heating or trap-crystal defects interaction models. A paper with the results obtained in this experiment "On the peculiarities of peak 4 in LiF TLD-100" has been submitted for publication to Nuclear Tracks and Radiation Measurements.

d) An experiment has been started with the purpose of determining the incidence of the sensitivity changes that occur during exposure on the measurement of environmental doses with TLD-100. Exposure intervals ranging from 15 days to 6 months are employed. The final objective of the experiment is to try to establish either a reliable correction procedure or to quantify the contribution of these sensitivity changes to the final measurement uncertainty. The intention is to collect TL data obtained during the four seasons of the year, i.e. obtained at different ambient temperatures and compare these dose data with those given by a calibrated Reuter-Stokes ionization chamber taken as reference instrument. The chamber is placed near the TLDs and is controlled by a computer where the dose data are continuously recorded, stored and analysed.

TL dosemeters include four different groups, of five TLD-100 detectors each, exposed together inside PMMA phantoms at the depth of $1 g/cm^2$. Three of the groups received, in addition to ambient doses, a calibration dose of 0.2 mGy (γ ^{60}Co), one group at the beginning of the exposure interval, another in the middle, and the remaining group at the end of the exposure. The fourth group only integrated ambient doses. In this way it is possible to follow the glow curve evolution during exposure and estimate sensitivity variations. From the data collected up to now (winter and spring) an increase in detector sensitivity during exposure can be appreciated, instead of fading.

Another interesting fact that surely deserves further attention is that independently of sensitivity changes, TL dosemeters tend to give a systematically lower ambient dose estimation than the Reuter-Stokes reference chamber (10-20%). As the TLDs and the chamber have been calibrated with gamma radiation in the same, well traced laboratory, this discrepancy cannot be attributed to inconsistencies in the gamma calibration of both types of dosemeters. A possible explanation, that should be confirmed, would be that the TL miniphantom thickness is not enough for the adequate measurement of the harder components of ambient radiations, especially the cosmic component. An additional experiment involving the exposure of TLDs under different thicknesses has started recently. This experiment needs to be complemented with exposures over water at the RISØ Field Station, to obtain careful data of the TLD-100 cosmic response.

e) During the opening session of the RISØ Monitoring Field Station some types of electronic dosemeters employed at CIEMAT were exposed to certified gamma sources. From these preliminary measurements an acceptable measurement accuracy was appreciated when enough resolution in the μGy range was provided by the dosemeters.

Publications

During the reporting period some of the papers formerly presented to scientific meetings or symposia have been published.

- Glow Curve Analysis: a method for improving TLD reliability. A. Delgado and J.M. Gomez Ros. Radiat. Prot. Dosim. 43 143-146 (1992).

- Temperature effects in LiF TLD-100 based environmental dosimetry. A.Delgado, J.M. Gomez Ros and J.L. Muñiz. Radiat. Prot. Dosim. 45 101-105 (1992).

- Confirmation of the evolution of TLD-100 glow peaks 4 and 5 during storage at ambient temperatures. A. Delgado, J.M. Gomez Ros ,J.L. Muñiz, A.J.J. Bos and T.M. Piters. Radiat. Prot. Dosim. 47 231-234 (1993).

- High energy photon response of different environmental TLDs. J.C. Saez-Vergara, J.M. Gomez Ros and A.Delgado. Radiat. Prot. Dosim. 47 327-330 (1993).

- A glow curve analysis method for non-linear heating hot gas readers. J.M. Gomez Ros, J.L. Muñiz, A. Delgado, L. Bøtter-Jensen and F. Jørgensen.Radiat. Prot. Dosim. 47 483-488 (1993).

Head of project 4: Dr.F.Pernička (Institute of Radiation Dosimetry, Prague)

I. Objectives of the reporting period

The aim of a research work done at IRD during the reporting period was divided into following tasks:

I.1. Development of software for deconvolution of Vinten SOLARO thermoluminescence glow curves using a TL model with a general order kinetic.

I.2. In flight measurements using both passive as well as active methods and subsequent study of the responses of some of the detectors to cosmic rays.

I.3. Environmental measurements at a Natural Environmental Monitoring Station (NEMS) at Risoe together with a final evaluation of the experiment SAZAVA.

II. Objectives for the next reporting period

During period September 1, 1993 - May 31, 1994 activities will be devoted into:

II.1. Installation of CIEMAT's deconvolution software into SOLARO system and its testing and use for TL studies. Different deconvolution techniques will be used for evaluation of LiF:Mg,Cu,P as a new material for the environmental dosimetry.

II.2. Analysis of in-flight measurements will continue. Responses of environmental dose and dose rate meters mainly to the ionizing part of cosmic radiation will be studied. Values for aircrafts can be used as the limiting estimates of the on-earth doses for persons living at high altitudes.

II.3. Comparative measurements of the environmental radiation fields in the Czech Republic will be organized. All measurement sites will be characterized by a spectral distribution of ambient photon radiation. Long term measurements with a plastic detector at the NEMS are planned.

III. Progress achieved including publications

Ad I.1. Deconvolution of TL glow curves

Program CONV was written to convert data stored in Solaro's files *solinfo.dat* and *solindx.dat* into ASCII format so that they can be handled by a number of commercial as well as user written packages. It is a part of the package DEKON developed for deconvolution of TL glow curves. The main program is written in Fortran and besides routine CONV it is using minimization system MINUIT. The model used to describe the TL process can be defined by a user. Deconvolution was tested on CIEMAT's data for LiF TLD-100. The glow curve together with its components unfolded using DEKON with a general order kinetics model is shown in Fig.1. Calculated activation energies for different peaks are in a good agreement with those obtained at CIEMAT. The whole package and its testing is described in /1/.

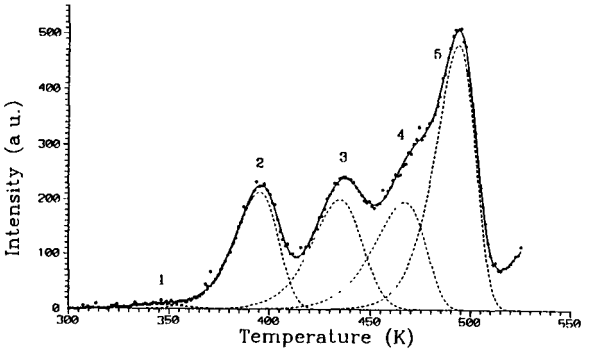


Fig.1. TL glow curve with unfolded components using a general order kinetic for TLD-100

Ad I.2. In-flight measurements

There are several methods available for the dose or dose equivalent determination from cosmic rays. Measurements of an ionizing component are usually performed with high pressure ionization chambers (HPIC), plastic scintillation detectors (PSD) and thermoluminescent detectors (TLD). Different moderator type instruments and bubble detectors are used for measurement of the neutron component and finally tissue equivalent proportional counters are used for straightforward measurements of the total dose equivalent.

Our experiments in a radiation field behind the shielding of a 600 MeV proton accelerator indicate negligible sensitivity of HPIC and PSD to neutrons with energy distribution such that about 40% of dose equivalent comes from neutrons between 20-350 MeV. This supports an idea that these instrument measure just the ionizing component of cosmic radiation.

The main contribution into an air ionization at investigated flight levels comes mainly from electrons and protons. Contributions from other types of radiation were neglected in our calculation of relative responses for HPIC and PSD to the ionizing component of cosmic radiation. The relative response (normalized to the response for Cs-137) to the ionizing component for HPIC and PSD was calculated as $R_{(HPIC)} = 1.091$ and $R_{(PSD)} = 0.882$. The 20% enhancement due to the transition effect for HPIC and particle fluxes and spectral distributions for middle latitudes were considered during calculations . The ratio of these figures is in good agreement with our experimental findings. The above responses were further used for analysis of our in flight measurements. The procedure is described in /2/. The latest measurements with LiF:Mg,Cu,P on the route Prague-Tel Aviv-Prague also show that this material is very suitable for these types of measurements due to its high sensitivity and this will be further studied.

Ad I.3. Comparative measurements of the environmental radiation at different sites

Total 17 institutions from former Czechoslovakia, one from Austria and one from FRG took part in the SAZAVA intercomparison organized by IRD Prague at the end of 1990. Together they used 24 instruments based on 4 different measuring principles. The selected experiments enabled calibration as well as measurement in different environmental conditions (different ratio of terrestrial to cosmic radiation). The measured values for different experiments are shown in Fig.2. The whole intercomparison and its evaluation is fully described in /3/.

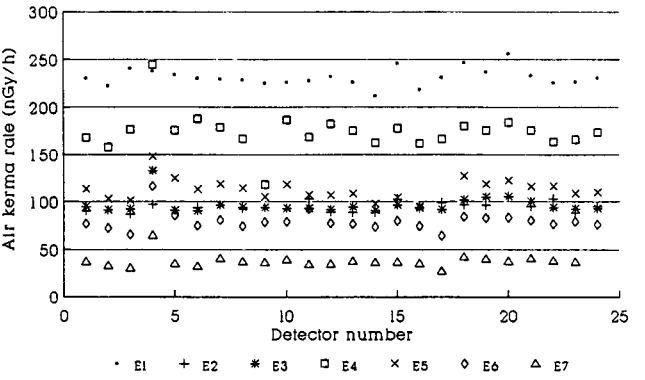


Fig.2. Corrected results for different experiments (E1-E7)

Besides of evaluation of these experiments the IRD group also participated in environmental radiation measurements at Risoe. Both intercomparisons proved the necessity for using similar procedures. They are especially important for former Central and East European Countries. It is believed that they can considerably improve the standard of measurements there.

Publications

/1/ Pernička F., Šlachta V. *System DEKON for evaluation of TL glow curves*, IRD Report, Prague, (1993), (in print)

/2/ Michalik V., Pernička F., Spurný F. *Some Aspects of the Exposure of Aircraft Crew Members to Cosmic Radiation*, Workshop on Individual Monitoring of Ionizing Radiation: The Impact of Recent ICRP and ICRU Publications, Villigen, May 5-7, (1993) (will be published in Radiat.Prot.Dosim.)

/3/ Pernička, F., Lauterbach, U., Pessara, W., Strachotinsky, C., Spurný, Z., Kažimír, D. *Intercomparison of Environmental Dose and Dose Rate Meters*, Joint Report of PTB and IRD, (1993), (in print)

Progress Report

Contract: **FI3P-CT920026** **Sector: A12**

Title: Detection and dosimetry of neutrons and charged particles at aviation altitudes in the earths's atmosphere.

1)	McAulay	Univ. Dublin
2)	Tommasino	ENEA
3)	Schraube	GSF
4)	O'Sullivan	DIAS
5)	Grillmaier	Univ. Saarlandes
6)	Spurný	IRD

I. Summary of Project Global Objectives and Achievements

The Project Global Objectives for the contract are summarised as:

1. To obtain detailed information on the flux and energy spectrum of neutrons at aviation altitudes.
2. To obtain charge spectra and flux for high Z particles at aviation altitudes.
3. To use the measured data to calculate the doses received by aircrew as a result of their occupation.
4. To validate and modify computer programs for the computation of doses on the basis of the impinging external radiation fields.
5. To suggest procedures to minimise doses.

During the first phase of the contract, progress has been good towards achieving the objectives listed above. Much of the equipment for making experimental measurements has been assembled and tested as described in the relevant reports of the individual Contractors. The testing has been greatly assisted by the facilities available at the CERN laboratory in Geneva and the addition of Dr Hofert as a Contractor earlier this year has usefully extended the expertise available for the Contract; his group has been able to provide well specified radiation fields for use in instrument calibration.
Experimental measurements have started aboard commercial flights and one balloon flight carrying track etch detectors has been made. it is hoped that it will be possible to carry out some measurements aboard supersonic passenger aircraft in the next phase of the contract.
Validation of computer codes for dose computation on the basis of experimental measurements is in progress and appropriate modifications will continue as more information becomes available on the particle energy spectra.
Discussions on methods which may be available for the routine assessment of doses within the aviation industry have taken place, particularly within the context of EURADOS Working Group II and it appears likely that it will be possible to make recommendations which will be appropriate for the recording of doses received by air crew under normal operational conditions.
It is expected that more rapid progress will be made in this section of the Contract when experimental data on doses becomes available.

Two new groups have been added to the Contractors during 1993 and the valuable contribution by Dr Hofert has been mentioned above. Dr Spurny of the Prague Institute of Radiation Dosimetry is the second new Contractor and has brought to the project considerable earlier experience in the field which will greatly assist in achieving the objectives of the Contract. He will be making experimental in-flight measurements and contributing to the microdosimetry and radiation protection interpretation of data with the help of track structure theory.
Two Contractors' meetings took place during the period relevant to this report and these have proved most valuable in initiating co-operation between the groups involved in different aspects of the Contract and in ensuring a mutual understanding of the overall problems involved.
The next period of the Contract should see the conclusion of the experimental measurements and the establishment of methods of determining doses on commercial airline routes. It will then be possible to decide on the best means for routine assessment of doses and on methods for minimizing the doses to flight crew in the aviation industry.
The work programme for the project has been well maintained to date and it is expected that it will be possible to achieve all of the objectives of the Contract by its completion date of May 1994.

Head of project 1: Dr. McAulay

II. Objectives for the reporting period

The objectives for this reporting period were:
1. To assess the radiological implications of in-flight exposure for air-crew employed by commercial airlines.
2. To consider the various options which might be available for the routine determination of in-flight doses and dose rates.
3. To consider methods which might be used to minimise doses received by air-crew under normal working conditions.
4. To investigate the need for appropriate briefing of air-crew in respect of in-flight exposure.

The objectives for the next and final period of the Contract are to complete the assessment of doses by air-crew on a number of typical routes; to produce recommendations for the monitoring and recording of radiation doses received by air-crew; to recommend, if appropriate, methods for minimising doses resulting from radiation exposure at aviation altitudes and to prepare in outline a list of points which could be included in information provided to air-crew employed within the European Community.

III. Progress achieved including publications

Considerable assistance in the progress of these aspects of the Contract has been provided by the establishment by the EURADOS Committee of a Working Group (WG II) specifically to consider Radiation Exposure of Aircrew. This has led to a broad range of information on scientific work in this area outside the Contract and to useful discussions with many of the members of the Working group.
The final conclusions relating to most of the objectives of this section of the Contract are dependent on the experimental measurements and dose assessments determined by the other Contractors. However, the interim conclusions based on the work carried out so far are as follows:
1. Air-crew on long haul flights at altitudes reaching more than 9,000 metres will receive considerably more radiation dosage in a typical working year than the dose limit recommended for members of the public. On such flights which take place at high latitudes, there may be the possibility that the dose limit for pregnant women could be exceeded. If this is established by the data obtained in the measurement programme, regulatory measures to protect such workers will be required.
2. Detailed consideration of the options for routine assessment of in-flight doses lead to two practical possibilities: individual monitoring either of the workplace or of the individuals concerned, or dose assessment on the basis of routes

and hours flown with check measurements at regular intervals. At present, the evidence favours the second of these options and if the measurements confirm the reproducibility of data then the allocation of a dose value for individual routes should be possible.

3. Doses received by air-crew could be minimised by varying the working schedule of individual members between different routes and duties. Detailed information on flight hours appears to be difficult to obtain within all Member States of the Community and it has not yet been possible to establish how collective dose reductions might be achieved in this way.

An attempt has been made with airline co-operation to look at collective dose reductions that could be obtained by varying altitudes and routes on the basis of existing dose data. The financial cost of such dose savings have been established to be prohibitively high and it is not intended to investigate this possibility further.

4. It is clear from discussions with air-crew from a number of different companies that there is a widespread concern over the question of in-flight radiation exposure. This concern is particularly strong in the case of female crew in relation to pregnancy. There is a clear need for appropriate information to be provided and the final report of this Contract will attempt to provide a framework indicating the areas of concern to flight crew.

Head of project 2: Dr. Tommasino

II. Objectives for the reporting period

Response evaluation of a multidetector system for short-term and long-term measurements of in-flight neutron exposures formed respectively by:
-Bubble detectors;
-Electrochemically etched track detectors of neutron recoils;
-Spark counter of damage tracks of neutron-induced fission fragments in Bismuth, Gold and Tantalum;
-Extended Rem-counter (investigated by Prof. M. Pelliccioni, INFN, Frascati, Roma).

For the next reporting period, the evaluation of the response of the multidetector system versus the neutron and proton energy will be continued, with particular regard to fission track and polycarbonate detectors.
Since in the first report period, only the detector based on bismuth was studied, new radiators (such as gold and tantalum) will also be investigated.
Special efforts will be devoted to the newly developed combination-detector formed by the bubble detector and its containing bottle, which has resulted in an attractive multidetector system. Field studies will be started, in which the passive detectors and the extended rem-counter will be irradiated simultaneously to investigate their relative response.

III. Progress achieved including publications

Extensive investigations have been carried out to study the response of track detectors of neutron-induced fission-fragments in bismuth. A novel counting procedure has been developed, by which it is possible to reduce the background by a factor of 1000.
Sufficiently high sensitivity for in-flight measurements of high energy neutrons can be achieved with this new counting technique, if long exposure times and large detector areas are used.
The bismuth fission track detector has been calibrated at different high-energy neutron and proton beams. Using the EURADOS Joint Irradiation Programme, calibration has been obtained by 46 and 66 MeV neutron beams from Paul Scherr Institute (Switzerland). Thanks to the support of Dr Francis Spurny , several calibrations have been carried out using different high energy proton beams from Dubna. At this stage of development, a Lower Limit of Detection-L.L.D. of about 0.1 mSv for neutron with energy above 50 MeV can be easily achieved. These results are of interest for the solution of the controversy existing with regard to the contribution of high energy neutrons to the in-flight aircrew exposure.

The Bubble dosimeter consists of a small polycarbonate bottle containing superheated freon droplets interspersed in an elastic polymer. In this first part of the project, it has been proved that the polycarbonate bottle (i.e. the container of the bubble detector) can be successfully used as track detector, which can be easily etched electrochemically. The combination formed by a bubble and damage track detector in one single device exploits the two most important developments, made in the past 20 years, in the field of fast neutron dosimetry. Furthermore, in spite of the fundamental differences in detection principle, the manual and/or automatic counting methods for electrochemically etched tracks and neutron-induced bubbles can be made using the same apparatus. In this first project period, an automatic counting system has been set up, which, although first developed for the counting of neutron induced bubbles, can be conveniently used also for the counting of etched tracks on the surface of polycarbonate bottles.
Finally, the response of polycarbonate bottles has been thoroughly investigated for neutron energy up to 30 MeV.
The characteristics of this novel neutron dosimeter have resulted as in the following:
-Response independent on the neutron incident angle;
-Possibility to identify the neutron incident angle;
-Response with no fading up to years of exposure;
-Reproducibility of the background;
-Lower Limit of Detection of 0.1 mSv;
-Response relatively independent on the neutron energy from 1 MeV up to at least 30 MeV;
These data add new advantages to the already attractive characteristics of polycarbonate neutron dosimeter in high-energy-accelerator fields and thus in cosmic ray fields.

For what concerns the extended rem-counter, Prof. Maurizio Pelliccioni has set up a self-powered monitoring system as required for in-flight neutron dosimetry. This new rem counter is now being calibrated in different fields, including the high energy irradiation facilities made available recently at CERN.

Publications:
Z. Lounis, M. Cavaioli, L. Tommasino and G. Torri (1993). Polycarbonate bottles and/or containers of bubble detectors as neutron dosimeters. To be published.
L. Tommasino (1993). Importance of track detectors in radiation protection dosimetry. Nuclear Tracks. In press.

Head of Project 2: Dr.H.Schraube
Scientific technical staff: Dr.W.Heinrich (subcontractor), Dr.J.Jakes, Dr.G.Leuthold, Dr.R.Meckbach, V.Mares, G.Schraube, E.Weitzenegger

II. Objectives for the reporting period

1. Dosimetric and spectrometric experiments in relativistic radiation fields
2. Response calculations of dosimetric devices
3. Calculation of cosmic radiation fields

III. Progress achieved including publications

Ad 1. Experiments in relativistic radiation fields

The group participated in the joint experiment at the CERN super proton synchrotron which was initiated and conducted by the CEC in the frame of this cooperative contract. The radiation fields behind two different shielding arrangements were provided and were intended to simulate to a certain extent cosmic ray fields. The fields may serve for a better understanding of the data measured during air borne experiments which suffer necessarily from poor statistics. In figure 1 the preliminary results of measurements with a tissue equivalent proportional counter (TEPC) is shown which simulates a tissue diameter of 2μm: While the dose (per particle on the target) is very similar behind both the iron and the concrete shield at LET lower than about 10keV/μm, a considerable additional dose contribution is observed between 10 and 100keV/μm for the iron shield condition. Considering the spectral shape, this may be attributed to recoil protons deliberated by neutrons in the energy range below 2MeV.

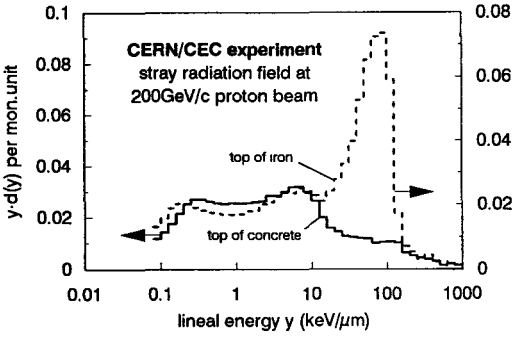


Figure 1: Relative dose distribution versus lineal energy as measured at the CERN relativistic stray radiation fields behind concrete (——) and iron (---)

Ad 2. Response calculations

Calculated cosmic radiation fields at aviation altitudes are to be validated experimentally, in particular by measurements with moderating devices and TEPC's in fligths on aircrafts. For this purpose it is necessary to calculate the response of the devices to each of the radiation field components, for the energy range of the expected spectral distributions.

Bonner spheres. A spectrometer consisting of a set of Bonner spheres is suitable to determine the spectral fluence distribution as the primary interesting quantity for neutrons, from which all other quantities may be derived. In order to study the response beyond 20MeV, the MCNP Monte Carlo calculations at the GSF were extended to 100MeV. In figure 2, the resulting 3-dimensional response matrix is shown. It is obvious that beyond 10MeV, the response drops considerably even for large spheres (18inch≈50kg). The conclusion is that for the neutron energies in the 100MeV range which are expected from theoretical considerations, additional devices are required if the full energy range is to be detected.

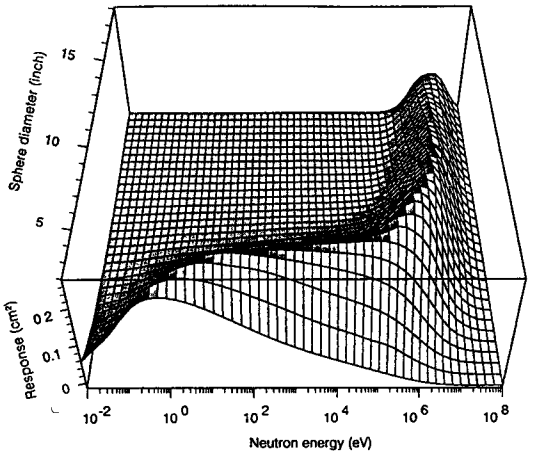


Figure 2: Three-dimensional representation of the response matrix of the studied Bonner sphere system up to 100MeV neutron energy (Mares and Schraube, 1993)

Tissue equivalent proportional counters. These calculations are being made by Monte Carlo simulation of the particle transport and energy deposition in the counter. The computations are being based on the PARTRAC analog photon-electron code (Henss, 1992). The code is endowed with a combinatorial geometry package, with which the counter geometry can be simulated in full detail, so that absolute microdosimetric lineal energy distributions for the different radiation field components and primary energies can be computed, taking also wall effects into account. The computations will be performed for counters of different shapes and wall thicknes, allowing for comparisons with measurements of different types of proportional counters.

An example of results of calculations performed with this code is shown in Figure.3, where calculated microdosimetric spectra are compared with experimental results for a cylindrical TEPC, for two primary photon energies.

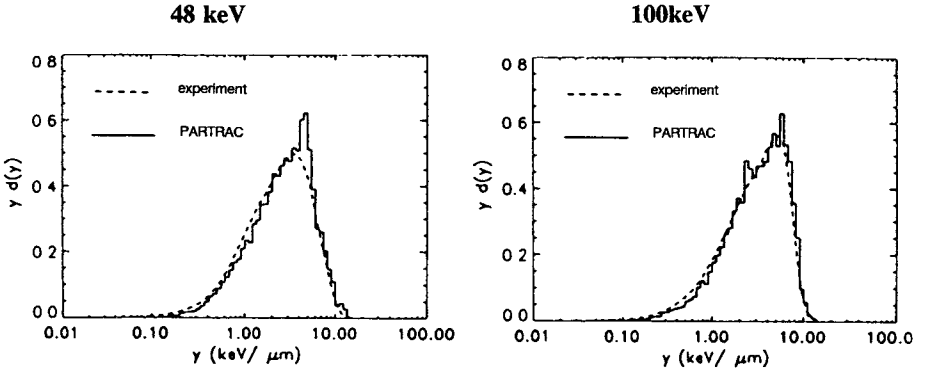


Figure 3: Comparison of calculated and measured microdosimetric lineal energy distributions, for primary photons with energies of 48 keV and 100keV. The experimental data were obtained with a cylindrical TEPC (KFA-JADE counter)

for a simulated tissue diameter of 2 μm (Olko et al., 1989), the computations performed with the PARTRAC code (S. Henss, 1992).

In its original form, the PARTRAC code allows to simulate electron track structures in the energy range from 10 eV to 100 keV. A semiempirical analytical model for secondary electron production cross sections is being scrutinized and will be implemented in the code, contributing to extend its energy range to relativistic energies. In order to reduce computing time, consideration is being given to the question of using condensed history Monte Carlo methods to simulate the electron transport in the non-active parts of the counter.

Finally, the extension of the PARTRAC code to include the simulation of particle transport and energy deposition for muons, primary and secondary heavy charged particles (protons, α-particles and ions), pions and neutrons is being studied.

Ad 3. Calculation of cosmic radiation fields.
The program LUIN has been used in the past in several studies to predict energy spectra and dose of cosmic rays and their secondaries at air flight altitudes. Although LUIN has been continously updated and improved over the years, the validity of its predictions is limited due to the fact that LUIN uses approximations to provide a fast computer code based on analytic solutions of the tranport equation. We have started a study to compare secondary particle production as described by LUIN with predictions of Monte-Carlo programs developed at CERN. Figure 4 shows as an example differential energy spectra of neutrons behind 10g/cm^2 of air calculated by LUIN and by the code GEANT/GHEISHA using the same cosmic ray proton spectrum as input. These results show reasonable agreement, however for larger depth of atmosphere, differences are observed which need more detailed studies.

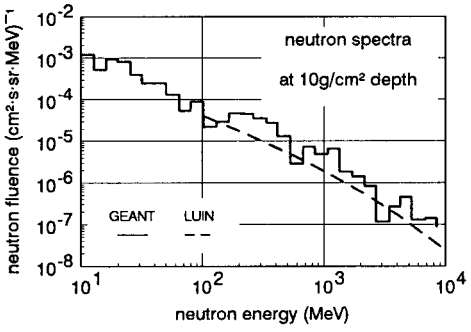


Figure 4: Differential energy spectra of neutrons at 10g/cm^2 depth of atmosphere calculated a) by LUIN (---) and b) by GEANT (histogram) for the same spectrum of primary cosmic rays.

References
P.Olko, Th.Schmitz, K.Morstin, A.Dydejczyk and J.Booz, Radiat. Prot. Dosim. 29, 105-108, 1989
S.Henss, PhD-Thesis, Techn.Universität München (1992)

Publication
V.Mares and H.Schraube: Evaluation of the response matrix of a Bonner sphere spectrometer with ^{6}LiI detector from thermal energy to 100MeV. Accepted for publication in Nucl.Instr.Meth.Radiat.Res. A (1993)

Head of project 4: Dr. O'Sullivan

II. Objectives for the reporting period

1. Investigate the suitability of the various available charged particle detectors for heavy ions and acquire quantities of the appropriate material.
2. Make arrangements for the calibration of detectors at a suitable particle accelerator.
3. Negotiate to secure exposure facilities on board commercial and other aircraft operating within the altitude region of interest.
4. Set up suitable processing and measuring equipment for the research program.
5. Make preliminary calibration measurements.
6. Investigate the possibility of collaboration with other groups involved in the project.

Objectives for the next period

1. Secure further exposure facilities on board commercial aircraft operating at higher altitude than that already under scrutiny.
2. Retrieve detectors currently being exposed for preliminary analysis.
3. Process sample of those detectors exposed, compile and analyse the data acquired and decide future of remaining stacks.
4. Set up collaboration with Dr. Tommasino's group at ENEA (Rome). Test out detection methods jointly by use of balloon exposure in Canada.
5. Negotiate and arrange for exposure on a Concorde aircraft to obtain data at the highest commercially travelled route.
6. Preparation of extra detector material and exposure of same at an accelerator for future exposures.

III. Progress achieved including publications

Arrangements were made in October '92 to have CR-39 detectors manufactured by Tasktrack in Bristol, U.K. and by American Acrylics in Conneticut,USA. Stacks were prepared and were ready for use by early December '92. Lexan stacks were also prepared.
Calibration exposures were carried out in December '92 at the Berkeley Bevalac. Stacks were exposed to Calcium and Iron nuclei at approx. 300MeV/N and 500MeV/N respectively.
A measuring system has been set up incorporating a high precision optical microscope with a Heidenhain transducer and display unit. Processing facilities are also being extended.
A number of sheets from both the CR-39 and Lexan stacks were processed to determine beam locations and energies. Initial calibration measurements have been made for Iron nuclei in Lexan.
Exposure facilities were secured for six detector stacks on board an Aer Lingus Boeing 747 aircraft operating on the Dublin - New York route. Two more detector stacks are being exposed on board the Irish Government Gulfstream Jet. Exposure of the detectors began in May '93 and all six stacks will remain in place for a period of several months.
Negotiations are proceeding satisfactorily with British Airways to expose detectors on Concorde at some later stage.
A set of detectors was prepared for exposure on a balloon flight in Canada, arranged through the courtesy of the US National Scientific Balloon Facility, scheduled for Aug.- Sept. 1993. It is hoped to use this exposure to carry out preliminary tests on a joint Dublin-ENEA(Rome) method for particle detection.

Head of project 5: Professor Grillmaier

II. Objectives for the reporting period

(a) Objectives for this reporting period
To calibrate the HANDI (Homburg Area Neutron Dosimeter) which is based on the use of low pressure tissue equivalent proportional counters (TEPC) in fields being of interest in civil aviation radiation protection: (i) heavy ion fields with Z 30 (GSI, Germany); (ii) radiation fields with spectral distribution similar to those encountered at altitudes of about 10-13 km (CERN, Switzerland).
To investigate the dose equivalent response of HANDI in neutron fields with high energy above 20 MeV.

(b) Objectives for the next reporting period
To measure dose equivalent and LET distribution in flight.
To continue the calibration of HANDI and to determine the dose equivalent response of HANDI by means of measurements and calculations in heavy ion fields and in fields similar to those found at flight altitudes.

III. Progress achieved including publications

The ambient dose equivalent meter HANDI developed in our laboratory is a portable, battery powered system which is equipped with a tissue equivalent detector (TEPC) simulating a small volume of human tissue. Using microdosimetric principles the HANDI provides absorbed dose, dose rate and lineal energy distribution. Furthermore, the actual value of dose equivalent is calculated in real time. Recently a cylindrical TEPC has been constructed in the laboratory for use with the HANDI. The detector designed for routine work in radiation protection has a large volume which provides sufficiently high sensitivity for radiation protection measurements in environments with low dose rate.
In the reporting period two experiments with the HANDI have been performed in heavy ion fields at the accelerator facility in GSI (Gesellschaft fur Schwerionenforschung) (D): (i) using a commercial spherical TEPC, nickel ions with energy of 650 MeV/u were measured; (ii) measurements with the new developed cylindrical TEPC have been performed in field of neon ions with energy of 400 MeV/u. Due to the very high intensity and energy of heavy ion radiation the preliminary measurements of nickel ions aimed at the assessment of the capability of the HANDI for calibration in high energetic heavy ion fields. The measurements in nickel ion fields were performed under parasitic circumstance, therefore it is not possible to report

quantitive results. However, the experiment shows that the HANDI is able to measure single energy deposition events of heavy ions and to provide spectral information about the radiation field.
In the 400 MeV/u neon ion field the HANDI was used under regular circumstance to measure absorbed dose and LET distribution (Fig 1). Because of the very high energy of neon ions it cannot be avoided to measure primary radiation ("PR" in Fig 1) which contributes more than about 50% to total measured absorbed dose. However, the peak of the distribution is in good agreement with calculated mean energy loss of primary beam of about 30 keV/ m. In the region of high LET (100 keV/ m) heavy particles from the interaction between wall materials and neon ions are detected ("SP"). The dose contribution of gamma radiation is minor (about 3%).

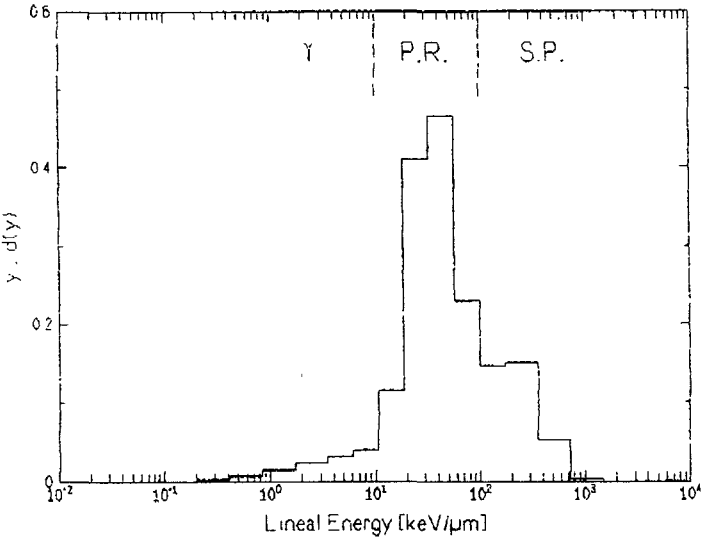


Figure 1: HANDI measurement in a neon ion beam with energy of 400 MeV/u at the GSI facility using the cylindrical TEPC. Below 10 keV/ m the dose contribution results mainly in gamma-radiation ( ), between 10 and 100 keV/ m of primary radiation (PR) and above 100 keV/ m of secondary particles (SP).

CERN in Geneva, Switzerland provides stray radiation fields which consist of positive hadrons (about 2/3 protons and 1/3 positive pions). In July 1993 an intercomparison with European dosimetric research groups took place. Experiments in the field of approximately 1 GeV/u hadrons with the HANDI using the cylindrical TEPC have been performed in order to determine dose equivalent and to provide informatio about the composition of the radiation field. The results of the HANDI measurements were used as reference for the other instruments. The evaluation of these experiments is not yet finished. In

order to achieve a more detailed analysis of the LET distribution it is foreseen to continue these measurements for the next reporting period using our laboratory system which provides higher resolution of 360 channels in contrast to 16 channels of the HANDI.
The investigations of the dose equivalent response of the HANDI to high energy neutrons in collaboration with PTB are in progress. The experiments have been performed in neutron fields with energies from 40 to 70 MeV at the Paul Scherrer Institute in Villigen, Switzerland. The response of HANDI is determined using cylindrical and commercial spherical TEPCs. The measurements in the neutron field with energy of 50 MeV show that the response of the HANDI is little lower than one.

Scientific staff:
M Freyermuth, S Gerdung, T Lim

Other research group(s) collaborating actively in this project:
- Gesellschaft fur Schwerionenforschung mbH, GSI Darmstadt (Dr D Schardt) (D)
- Paul Scherrer Institute, PSI Villigen (Dr R Henneck, Dr P Schmelzbach)(CH)
- Physikalisch-Technische Bundesanstalt, PTB Braunschweig (Dr U Schrewe, Dr H Schuhmacher) (D)

Head of project 6: Dr. Spurný

II. Objectives for the reporting period

1. To contribute to the calibration of detectors in the beams of high energy particles and behind shielding.
2. To obtain further data on exposure levels during in - flight measurements and through the search and analysis of published data.

III. Objectives for the next reporting period

(August 1993 - May 1994)

1. To continue in the calibration of detectors and equipment in the beams of high energy particles and behind their shielding.
2. To continue to collect the data on exposure levels during in flight measurements.
3. To contribute to the microdosimetry and radiation protection interpretation of data with the help of track structure theory.

III. Progress achieved including publications

Ad 1.1. Calibration in high energy proton beams

High energy proton beams available at the Joint Institute of Nuclear Research at Dubna, Russia were utilized to calibrate TLDs' used for aircrew measurements at IRD AS CR. It was proved, that the response of TLDs' to protons with energies above several tens MeV is practically the same as the response to ^{60}Co radiation [2]. In the same beams, with six different proton energies from 94 to 238MeV two sets of six complexes SSNTD with radiators (Au, Bi, Ta) were irradiated for other participant at the project, Dr.L.Tommasino ENEA, Rome, Italy. Monitoring and fluence determination for these calibrations were ensured.

Ad 1.2. Calibration in lower energy charged particle beams

To ensure correct interpretation of TLDs' data, their response to lower energy charged particles should be known. Several series of such studies were performed for protons and alphas with energies up to 10MeV. Results obtained have shown [6] that the response of common TLDs' to such higher LET charged particles is generally less than to ^{60}Co radiation.

ad 1.3. Calibration in reference fields behind shielding of high energy particle accelerators

Several reference fields have been formed behind shielding of JINR high energy particle accelerators [9]. During reporting period, some calibration of detectors and equipment in these fields were performed.

SSNTDs' in contact with fission fragment radiators (Bi, Au, ^{232}Th) were tested both in these reference fields as well as in high energy charged particle beams. It was found out that this type of detectors is sensitive enough to be used for radiation protection purposes [7].

The most of studies has been performed in the reference fields behind the shielding of phasotron. BDNDs' were tested both in soft and hard reference field. Their response in soft reference field is comparable with the response to ^{252}Cf neutrons, their response in hard reference field (large contribution of high energy neutrons to the total equivalent dose) is substantially lower. The response of BDND to neutrons with energies about 100MeV would represent only about ~40% of their response to ^{252}Cf neutrons [8].

GM-based equipment RP114 for environmental low LET dose equivalent measurements was also tested in soft reference field at the phasotron. It was found out, that its response is higher (+~40%) as compared to C/CO_2 ionization chamber, probably due to the thermal neutron sensitivity of RP114 instrument. The response of RS112 ionization chamber was comparable with C/CO_2 chamber. Two series of calibration experiments were performed at the end of reporting period (July 23-27, 1993) behind the shielding at CERN. Their evaluation and analysis has just started.

Ad 2.1. Further in flight radiation exposure data acquisition

In total 20 other in-flight measurements have been performed during the reporting period on the board of CSA and other aircraft, during return flights from Prague to New York, Sophia, Stockholm (Umea), Madrid, Tel Aviv and Beirut [1,3,5, other publications in preparation].

Obtained data have confirmed the tendencies observed, i.e.:

- the important influence of flight altitude on the exposure level (increase by 10 to 15% for each 2000 feet); at 35000 feet total dose equivalent was typically about 8μSv per hour;
- practically no influence of geographic latitude for more than about ~45°N at Europe area (Umea ~66°N); the decrease of this level when flying from Prague (50°N) to the south (Sophia, Madrid) was observed.

New results have been acquired during these measurements:

- large surface CR-39 detector was exposed, its evaluation permitted to appreciate high LET particle fluence rate during PRG-JFK-PRG flight to 2.9×10^{-4} cm^{-2}.s^{-1};
- the influence of decreasing solar activity was already registered; exposure level at the end of 1992 was at the same altitude about 10% higher than at the spring 1991; it correspond well to neutron galactic cosmic network data.

Ad 2.2. Review of available spectral data

Spectral data available particularly from Russian sources were reviewed and analyzed. First attempt to interpret the measured data with these spectral data available in this way was published during CEC Workshop [10]. As an example, Fig.1 presents the contribution of neutrons of different energies to the total dose, resp. dose equivalent from neutrons at high (>45°N) latitudes. High energy neutrons (≥several tens MeV) contribute to the total dose equivalent more than by 50% (for equatorial region this contribution represents less than 10%). As far as protons, resp. electrons are concerned the contribution of high energy particles is also important (50% of dose equivalent due to protons with energies higher than about 500MeV, resp.due to electrons with energies higher than about 30MeV).

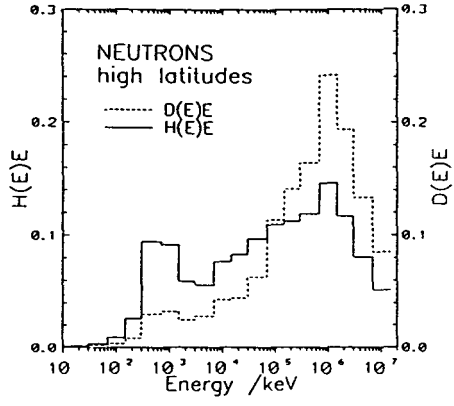


Fig.1:

The distribution of absorbed dose and dose equivalent for typical spectrum of cosmic ray neutrons at high latitudes. The areas under the curves are normalized to unity

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Progress Report

Contract: FI3P-CT920032 Sector: A12

Title: Dosimetry of beta and low-energy photon radiations.

1)	Christensen	Lab. Risø
2)	Chartier	CEA - FAR
3)	Francis	NRPB
4)	Herbaut	CEA - Grenoble
5)	Marshall	UKAEA
6)	Gasiot	Univ. Montpellier II
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8)	Charles	IITL

I. Summary of Project Global Objectives and Achievements

Objectives

Ultimate objectives of the project are to develop standard calibration facilities and to establish standardised measurement and calculation procedures for dosimetry of weakly penetrating radiations. This will lead to a more consistent and reproducible dosimetric practice for determining exposures to individuals throughout the EEC countries. The proposed research programme is expected to bring about new knowledge and developments in the following main areas:

- identification and evaluation of influencing parameters important for the dosimetric characteristics of the radiation fields in designing standard beta calibration beams, e.g. source construction, surrounding shieldings, and beam-flattening filters.

- characterisation of beta radiation and the accompanying photon radiation (bremsstrahlung and characteristic X rays) fields in terms of dosimetric quantities as well as in terms of energy and angular spectra.

- investigation and development of measurement techniques and procedures for characterising beta radiation (with the accompanying photon radiations) fields and for individual monitoring for doses from these fields.

- establishment and characterisation of low-energy photon radiation calibration beams and characterisation of individual dosemeters for dosimetry of these fields.

- development and validation of methods for the measurement and calculation of doses from radioactive particulates ("hot particles").

The results of this study will lead to the realisation and characterisation of beta calibration facilities which conform to the specifications of ISO Series 2 references as well as low-energy photon calibration facilities and thereby facilitate calibration of personal dosemeters and monitoring instruments for weakly penetrating radiations. Expected results of the project are furthermore development of improved dosimetry techniques and procedures for characterising weakly penetrating radiations and for personal dosimetry in such fields, as well as for measurement of doses from radioactive particulates ("hot particles").

Achievements

Development of beta calibration facilities

This project is aimed at identifying and evaluating influencing parameters important for the dosimetric characteristics of the beta radiation fields and using this information to define optimal designs of the irradiation facilities. Important influencing parameters are: source construction, surrounding shieldings and beam-flattening filters.
Work has continued in characterising beta radiation fields in terms of $\dot{D}_t(d;\alpha^o)$ from different constructions of sources. Data have been obtained for extended sources and comparisons have been made with data from ISO Series 1 (PTB-Büchler) secondary standard "point" sources. The dose rates measured for the extended sources (dimensions: square, 40 mm x 40 mm, and circular, 42 mm in diameter), are of the order of 10 to 100 times those produced by the "point" sources and significant differences are observed in the depth-dose curves obtained for the two types of sources. When beta radiation beams are used to determine dosemeter responses the source design used is therefore important for the results. Results obtained for a ^{14}C source consisting of a 0.9 mm thick acrylic sheet with the the ^{14}C radioisotope incorporated in the plastic material indicate that this type of source is capable of providing calibration fields that comply with specifications setup by the ISO for Series 2 sources. Computer programs have been developed for generating beta spectra in the modelling of beta sources and two sources have been modelled. The photon radiation emitted from an extended area $^{90}Sr/^{90}Y$ source have been studied by using lead absorbers. The contribution from the photon radiation to the total dose rate is less than 0.05% for distances up to 40 cm from the source.

Development and study of techniques and methods for characterising beta radiation fields and for individual monitoring for beta radiation doses

The aim of this work is development and study of different dosimetry techniques and procedures for use for characterisation of beta radiation fields and for use for individual dosimetry of beta radiation doses. Dosimetry techniques involved sofar are: extrapolation chambers, geiger tubes, solid state dosemeters and beta spectrometers. Furthermore computer programs have been developed for validation of the experimental results obtained by these techniques.

Extrapolation chamber

The extrapolation chamber measurement method is the basic standard dosimetry measurement method applied to beta dosimetry and computerised automated measurement procedures have been established in five of the laboratories participating in the project.
Important aims of studying the extrapolation chamber measurement method are to optimise the evaluation procedure and the accuracy of measurement of the method. Joint results have been obtained through comparative measurements of $\dot{H}'(d;\alpha^o)$ and the collected data have been used as basis for further refinement of the measurement method and evaluation procedure. The results from intercomparison programmes are reported in a separate section of this report.
Preliminary results obtained from measurements of transmission factors using a geiger tube indicate that this detector may give result very close to those obtained by the extrapolation chamber. The geiger detector would be an attractive alternative to the extrapolation chamber for such measurements due to a much simpler measurement procedure.

Thin solid state detectors

Solid state dosemeters intented for characterisation of beta radiation fields and for individual monitoring for skin doses from beta radiation should contain detectors with a small effective

thickness capable of measuring $\dot{H}'(d;\alpha^o)$ nearly independently of energy and angle of incidence of the radiation. Response data of thin TL detectors and TSEE detectors for exposures to beta radiations show that it is possible to obtain solid state detectors with satisfactory response characteristics. Beta response data obtained for BeO TSEE detectors show that they can measure beta radiation doses with an efficiency of 100%, as expected for a detector with a thickness of 100 nm. For thin TL detectors (carbon-loaded MgB_4O_7:Dy and LiF detectors and LiF:Mg,Cu,P thin GR-200 detectors) the efficiency for the measurement of beta doses from ^{147}Pm varied from about 50% to 90%. For the measurement of $H_p(0.07;\alpha^o)$ the lower efficiency can be compensated for by using a cover with a thickness lower than 0.07 mm tissue-equivalent material. Dose mapping based on the laser heating TLD technique and the OSL measurement method has been further developed. The methods have been used for measurements of dose distributions close to a small ("hot particle") ^{60}Co source and investigations are in progress for using the methods for the determination of dose homogeneities of beta calibration beams.

Beta spectrometry

Beta spectrometry will be used for obtaining a detailed knowledge of energy and spatial distributions of beta radiation fields. The results will be compared with calculated data. A computer program has been developed for determining energy spectra from modelled source constructions.
A beta spectrometer based on two silicon semiconductor diodes and provided with facilities for automated scanning of the radiation fields is being established at Fontenay-aux-Roses and will be operational within the next year.

Low-energy photon dosimetry.

An X-ray irradiation facility, operating in the high voltage range from 5 kV to 100 kV has been established at CEA Fontenay-aux-Roses for producing low-energy photons of energies in the range from 5 to 15 keV. The irradiation facility will act as a primary radiation source of low-energy X-radiation. Initial tests of the stability of the X-ray unit have given satisfactory results.

Hot particle dosimetry

The overall aim of the project is to develop and validate methods for the measurement and calculation of doses from radioactive particulates ("hot particles").
During the current reporting period test sources of ^{60}Co and ^{170}Tm with well-defined source designs have been produced. Comparative measurements of doses from a ^{60}Co source using different measurement techniques have been performed at different laboratories: Berkeley (extrapolation chamber and radiochromic dye films), NIST, Washington DC (radiochromic dye films), CEA,Grenoble (extrapolation chamber and TLD) and University of Montpellier (TLD and OSL). The measurements for all laboratories and for other sources are currently being carried out and collated.
Two calculational techniques have been used in this study: VARSKIN, a semi-empirical, PC-based, code which uses an integration of Berger point kernels, and a PC-based Monte Carlo code developed by Dr. Patau.
Good agreement was obtained between measured and calculated dose data for ^{60}Co when a modified VARSKIN code was used. However, calculations using the Monte Carlo code produced data significantly different from the measured data. Results obtained for the ^{170}Tm source showed good agreement between experimental and calculated data using both the modified VARSKIN code and a Monte Carlo code, except for doses averaged over large areas. The cause of observed discrepancy between calculated and experimental data is being further studied.

Intercomparison of Extrapolation Chamber Measurement Methods used by the Participating Laboratories

An intercomparison of measurements of dose rates from an extended ^{147}Pm source using the extrapolation chamber measurement methods established at laboratories of the participants of the contract (NRPB, CEA-Fontenay-aux-Roses, CEA-Grenoble, Nuclear Electric, and RISØ) has been initiated with the main purpose of ensuring consistency of the standard beta dosimetry exercised by the participants. Objectives of the intercomparison exercise were to cooperate in identifying and solving problems involved with the extrapolation chamber measurement method and especially in evaluating on the measurement uncertainty valid for the method.

An extended area ^{147}Pm source (active diameter 42 mm) was applied for the intercomparison. The source was kept in the standard French CEA type holder.

The measurement programme comprised the determination of the dose rates at distances of 15, and 20 cm from the source and at angles of incidence of the radiation of 0° and 45°. The results should be given in terms of $\dot{D}_t(0.02;\alpha^o)$ and $\dot{D}_t(0.07;\alpha^o)$ and furthermore depth-dose data should be acquired for the range from the thickness of the entrance window of the extrapolation chamber and up to 9 $mg.cm^{-2}$. Uncertainties on the reported dose rate values should be evaluated in accordance with procedures adopted previously. It was agreed that a number of systematic uncertainties would essentially be equal for all systems and could therefore be combined to a single value that could be used by all participants. The main uncertainty componant to be assessed by each participant would be that related to the determination of the slope of the extrapolation curve.

In this report only preliminary results will be presented as results are only available from three of the five participants of the exercise. When all measurements have finished the data will be evaluated and a complete presentation of the results will be given in the next progress report.

The main characteristics of the extrapolation chambers used for the measurements of dose rates presented in this report are given in Table 1 and the measured dose rates $\dot{D}_t(0.07;\alpha^o)$ and $\dot{D}_t(0.02;\alpha^o)$ are presented in Table 2 and Table 3. It can be seen that the results obtained by the three laboratories in general show satisfactory agreement.

Table 1. Main characteristics of extrapolation chambers used for measurement of dose rates presented in this report.

Laboratory	Chamber Type	Entr. window		Electrode		Electrom.
		Thickness ($mg.cm^{-2}$)	Material	Diam. (mm)	Material	Lower range
RISØ	PTW 23392	0.66	Mylar	30	Perspex	10^{-17}
CEA/FAR	PTW 23392	2.6	Mylar	30	Perspex	10^{-16}
NRPB	NRPB design	0.85	Mylar	≈30	MS20	10^{-17}

Table 2. Measured dose rates, $\dot{D}_t(0.07;\alpha^\circ)$ from the ^{147}Pm source at distances 15 and 20 cm from the source and for angles of radiation incidence 0° and 45°.
(Reference date: 1. January 1993).

Laboratory	$\dot{D}_t(0.07;\alpha^\circ)$ in $mGy.h^{-1}$.			
	15 cm distance		20 cm distance	
	$\alpha=0^\circ$	$\alpha=45^\circ$	$\alpha=0^\circ$	$\alpha=45^\circ$
RISØ	80.2	56.2	16.9	11.6
CEA/FAR	78.8	52.2	16.2	11.2
NRPB	78.4	53.7	15.4	11.3

Table 3. Measured dose rates, $\dot{D}_t(0.02;\alpha^\circ)$ from the ^{147}Pm source at distances 15 and 20 cm from the source and for angles of radiation incidence 0° and 45°.
(Reference date: 1. January 1993).

Laboratory	$\dot{D}_t(0.02;\alpha^\circ)$ in $mGy.h^{-1}$.			
	15 cm distance		20 cm distance	
	$\alpha=0^\circ$	$\alpha=45^\circ$	$\alpha=0^\circ$	$\alpha=45^\circ$
RISØ	182	153	44.3	36.9
CEA/FAR	179	145	43.9	35.8
NRPB	183	152	42.0	36.9

Head of project 1: Dr. Christensen

II. Objectives for the reporting period

- Characterisation of the radiation fields from an extended "infinitely" thick ^{14}C source.
- Refinement and standardisation of the extrapolation chamber measurement method, in particular, by participating in an intercomparison programme concerned with measurement of dose rates from an extended ^{147}Pm source.
- Development and analyses of thin thermoluminescent detectors for application for individual dosimetry of weakly penetrating radiations.

IIA. Objectives for the next reporting period

- Continuation of the work concerned with characterisation of the radiation field from extended ^{14}C sources
- Characterisation of the radiation field from a ^{63}Ni source.
- Establishment and calibration of a ^{55}Fe 6-keV photon radiation field.
- Evaluation in collaboration with other participants the results obtained from intercomparison programmes performed on ^{204}Tl and ^{147}Pm sources.
- Continuation of the work concerned with development and investigation of thin solid state dosemeters for dosimetry of weakly penetrating radiations.
- Continuation of the work concerned with the characterisation of the radiation field from a $^{106}Ru/^{106}Rh$ source.

III. Progress achieved including publications

1. Characterisation of the radiation field from a ^{14}C source

The source construction used for this study was a 220 mm x 220 mm x 0.9 mm perspex sheet with the ^{14}C radionuclide incorporated in the acrylic material. The source contains 18.5 $MBq.g^{-1}$ perspex with an emission rate of 2×10^4 s^{-1} cm^{-2} beta particles (2π geometry) from the surface of the source. The thickness of the source (equivalent to about 100 $mg.cm^{-2}$) is larger than the maximum range of ^{14}C beta rays and the energy spectrum obtained from this type of source is therefore that valid for an infinitely thick source and will be different from the un-moderated spectrum of the ^{14}C radionuclide. Shields of perspex, 1 mm thick, and with holes of different diameter from 30 mm to 150 mm, positioned in front of the source enabled different sizes of the source to be studied.

1.1 Residual maximum beta particle energy

Residual maximum beta particle energy, E_{res}, was determined for different sizes of the source and at distances of 5, 10 and 15 cm above the source centrum. The measurements were carried out with a geiger tube, LND type 7231 with an end window thickness of 1.60 $mg.cm^{-2}$, connected to an Eberline mini scaler type MS-2. Mylar foils were used as absorbers and scaling factors given by Cross[1] were used for converting to tissue-equivalent thicknesses. The evaluation of E_{res} from the

measured depth-dose data was made according to the procedure described in [2].
From the results presented in Table 1 it can be seen that the ISO requirement of $E_{res} \geq 0.09$ MeV is fulfilled for calibration distances up til approximately 15 cm for a 150 mm diameter source.

Table 1. Measured residual maximum beta particle ranges and the values of residual maximum beta particle energy evaluated from them for distances = 5, 10 and 15 cm. The results were obtained for a 150 mm diameter source.

Source to detector distance cm.	Residual maximum beta range, R_{res} $mg.cm^{-2}$	Residual maximum beta energy, E_{res} MeV
5	17	0.12
10	11	0.10
15	9	0.09

1.2 Influence of source area.

The area of the source influences the dose rate, dose homogeneity and energy spectrum of the radiation field. Sources with varying areas were obtained by shielding the source by 1 mm thick perspex sheets with holes of diameters from 30 to 150 mm.

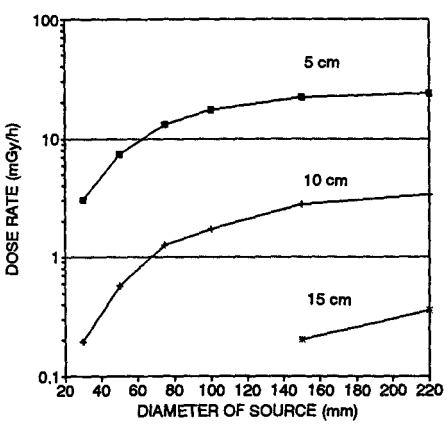

Figure 1. Dose rate as a function of diameter of source, measured for a ^{14}C source at distances: 5, 10, and 15 cm from the source and for 0° radiation incidence.

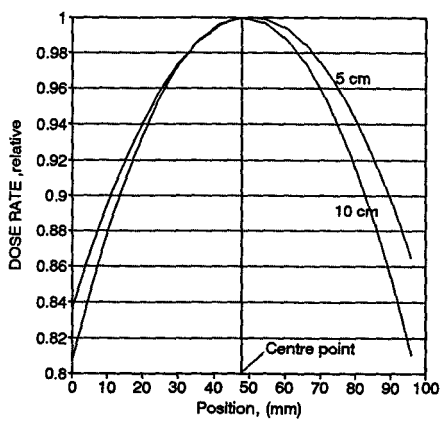

Figure 2. Dose rate homogeneity in planes at distances, 5 and 10 cm, below a 150 mm diameter ^{14}C source. Results are presented as curves obtained from 2. degree polynomial fits of experimental data.

Dose rate:
An extrapolation chamber with an entrance window thickness of 0,61 mg.cm^{-2} tissue-equivalent material and an electrode diameter of 30 mm was used for the measurement of dose rates. The results obtained for different areas of the source and for different distances from the source are shown in Figure 1.It can be seen that for calibration distances below 10 cm only a small increase of dose rate is gained by using a larger source diameter than 150 mm.

Dose homogeneity:
Analyses of the homogeneity of the dose rates of the radiation beam have been performed by use of 5 mm diameter, carbon-loaded MgB_4O_7:Dy TL detectors. The detectors show an effective thickness equivalent to approximately 2 mg.cm^{-2} and can therefore be expected to give a reasonable estimate of the dose rate measured with the extrapolation chamber having a window thickness equivalent to 0.61 mg.cm^{-2}. Initial results, shown in Figure 2, indicate that for a 150 mm diameter source a dose rate homogeneity within $\pm 10\%$, as required by the ISO, can be fulfilled over a beam diameter of 100 mm for calibration distances up to approximately 10 cm.

Beta energy spectrum:
Differences in the composition of the beta energy spectrum will be indicated by differences in the shape of the normalised depth-dose curves. Figure 3 shows such curves obtained for two distances from a 150 mm diameter ^{14}C source and for normal radiation incidence. For comparison results are also given for two ^{147}Pm sources for a distance of 20 cm from the source. Further measurements are in progress using other source sizes and angles of incidence of the radiation.

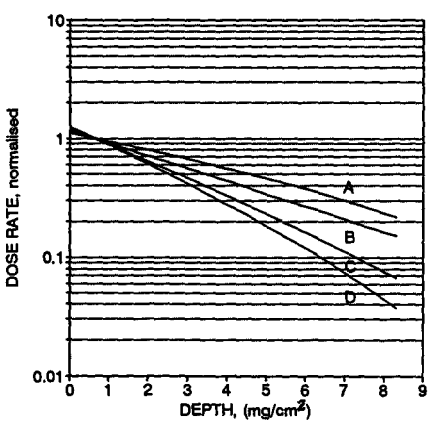

Figure 3. Depth-dose curves, normalised to depth 0.61 mg.cm^{-2}, obtained for a 150 mm diameter ^{14}C source and two ^{147}Pm sources. A: 42 mm diameter ^{147}Pm source at 20 cm. B: PTB-Büchler "point" ^{147}Pm source at 20 cm. C: ^{14}C source at 5cm and D: ^{14}C source at 10 cm. All data for 0° radiation incidence.

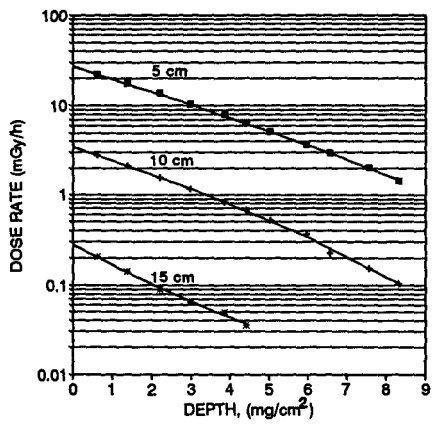

Figure 4. Depth-dose data obtained for a 150 mm diameter ^{14}C source at distances: 5, 10 and 15 cm from the source. Symbols indicate experimental values and lines fitted data using a (1+3) degree polynomial fit. Angle of incidence of the radiation : 0°.

1.3 Measurement of dose rate $\dot{D}_i(d;\alpha°)$.

Work is in progress for characterising the radiation field from ^{14}C sources of different sizes and at different distances from the source in terms of $\dot{D}_i(d;\alpha°)$. In addition to the existing source also a

source with minimal thickness of the radioactive layer will be included in the study. Figure 4 presents data of $\dot{D}_t(d;0°)$ obtained for a 150 mm diameter ^{14}C source at three distances from the source.

The extrapolation chamber is the basic instrument to be used for the measurement of $\dot{D}_t(d;\alpha°)$; however preliminary results obtained by using a geiger tube for the measurement of transmission factors for ^{147}Pm and ^{14}C sources indicate that for an absorption amounting up to about 90% of the total absorption the data obtained by the two methods show good agreement. The extrapolation chamber measurement method is complex and time-consuming, in particular, when low dose rates are involved, and it would be a great advantage if the geiger tube could be used for some of the absorber measurements. The geiger measurement method will be further studied for this purpose. Figure 5 illustrates the capability of the geiger tube to measure dose transmission factors.

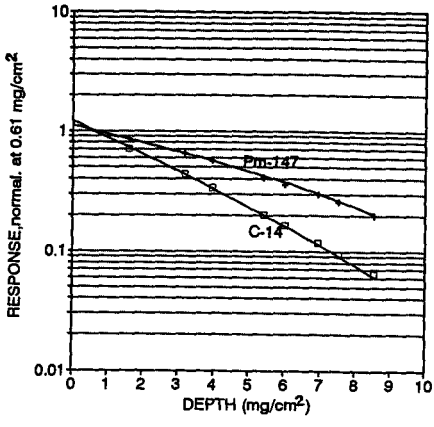

Figure 5. Depth-dose curves, normalised to depth 0.61 mg.cm^{-2}, obtained for a 150 mm diameter ^{14}C source at distance: 10 cm, and for a 40 mm x 40 mm ^{147}Pm source at distance: 20 cm. Radiation incidence: 0°. Symbols indicate experimental values obtained with a geiger tube and lines the fitted data obtained from extrapolation chamber measurements.

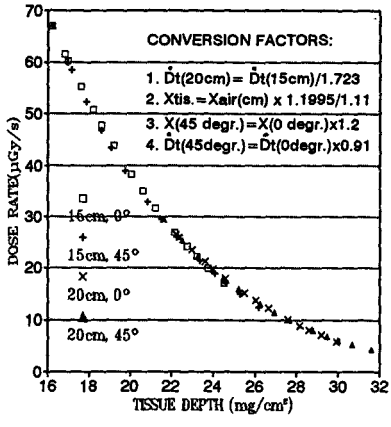

Figure 6. Depth-dose data obtained for a 42 mm diameter ^{147}Pm source at distances: 15 and 20 cm, and for angles of radiation incidence: 0° and 45°. Symbols indicate corrected experimental data (see text) and the line a polynomial fit calculated for all corrected data.

2. Participation in the ^{147}Pm intercomparison programme.

The results obtained by Risø from the participation in the ^{147}Pm intercomparison exercise have been discussed in the general joint progress report. In this report some further evaluation of the transmission data obtained by Risø will be presented.

Transmission data were measured for two distances: 15 and 20 cm, and for two angles of incidence of the radiation: 0° and 45°. Filters of mylar ranging from 0.8 to 9 mg.cm^{-2} were used for all four series of measurement. A factor of 0.92 (scaling factor) was used to convert from mylar thickness to equivalent tissue thickness [1].

Preliminary analyses indicate that all data from the four series of measurement can be represented by one 3rd degree polynomial expression if simple corrections are applied for change in geometry

due to change of distance from 15 cm to 20 cm, and for change in backscatter and absorber thickness due to change of radiation incidence from 0° to 45°. Furthermore the air mass between source and measuring point should also be counted as absorber mass and added to the mass of the mylar foils. The change in backscatter was measured by comparing values of $\dot{D}_t(0;0°)$ and $\dot{D}_t(0;45°)$. Corrections for change of geometry due to change of irradiation distance were obtained by comparing values of $\dot{D}_t(0;0°)$ for the two distances. Corrections for increase of absorber thickness with change of angle of radiation incidence from 0° to 45° were obtained by comparing values of $\dot{D}_t(d;\alpha°)$ for the two angles after corrections had been made for change of backscatter. Figure 6 presents the corrected experimental data obtained from the four series of measurement together with a polynomial fit of the data. The factors used for correcting the experimental data are also given in the figure. The expression for the data fit is:

$$\dot{D}_t(X) = 383.032 - 32.181 \times (X) + 0.9332 \times (X)^2 - 0.009311 \times X^3$$

where $\dot{D}_t$ is expressed in $\mu Gy.s^{-1}$ and X in $mg.cm^{-2}$ tissue-equivalent material.

The possibilities of characterising beta radiation fields in terms of $\dot{D}_t(d;\alpha°)$ by using only one polynomial expression and a few correction factors will be further studied.

3. Development and study of thin TL detectors.

Work has continued in studying the dosimetric characteristics of the Chinese LiF:Mg,Cu,P TL material. The material is commercially available as powder and in the form of thin detectors consisting of about 5 $mg.cm^{-2}$ layer of the TL crystals fixed onto a polyimid (kapton) tape (GR-200 thin detectors)[3]. The detectors are available in 5 mm x 5 mm and 10 mm x 10 mm sizes. For this investigation pieces of 5 mm diameter size were cut from the squared samples in order to get detector sizes fitted for the personal TLD dosemeter used at Risø. Similar thin detector samples were prepared from the TL powder by using a heat-resistant kapton tape. In order to make the detectors suited for automatic handling they were fixed onto Al or Teflon pieces, 0.7 mm thick and 5 mm in diameter, by means of a heat-resistant silicone-based glue.

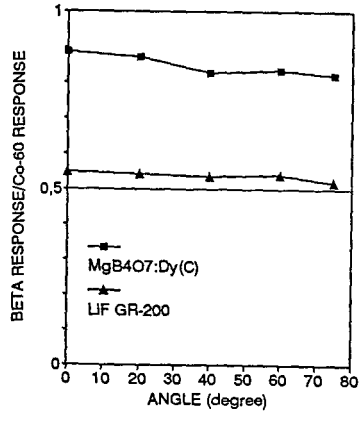

Figure 7. Energy and angular responses of LiF:Mg,Cu,P GR-200 thin detectors and MgB_4O_7:Dy carbon-loaded detectors for exposure to ^{147}Pm beta radiation at a distance of 15 cm from the source.

Figure 7 shows energy and angular responses of the GR-200 thin detectors and carbon-loaded MgB_4O_7:Dy detectors (from Boris Kidric Institute) for exposure to beta radiation from a 42 mm diameter ^{147}Pm source. Because of significant attenuation of the beta radiation occurring in the grains of the GR-200 detectors the response of the detectors is only 50% of that obtained from exposure to ^{60}Co radiation. By using a smaller grain size (less than 25μm) it was possible to increase the response to 65%[4]; however this was achieved at the expense of lower sensitivity resulting in a decrease of the ratio of the sensitivity Gr-200/MgB_4O_7:Dy from 4.3 to 1.8.

References and publications

1. Cross, W.G.,Variation of Beta Dose Attenuation in Different Media. Phys. Med. Biol.,13, 611-618 (1968).
2. ISO. Reference Beta Radiations for Calibrating Dosemeters and Doserate Meters and determining their Response as a Function of Beta Radiation Energy. ISO 6980. First Revision, 1990-11-01.
3. Wu Fang, Zha Ziying, Li Jian and Zhu Zhongmei, LiF(Mg,Cu,P) Thin dosemeter for skin dose measurement, Radiat. Prot. Dosim. 34, 331-334 (1990).
4. Christensen, P., Study of LiF:Mg,Cu,P TL Detectors for Individual Monitoring for Weakly Penetrating Radiations, Radiat. Prot. Dosim. 47, 425-430, (1993)

Head of project 2 : Dr. Chartier

II. Objectives for the reporting period.

The programme of work initiated by the CEA/SDOS involves 3 main topics which are considered in the reporting period. Those topics are:

a) the participation in an intercomparison of dose measurements in the radiation field of a ^{147}Pm source.The results have been distributed to the participants at the Contract meeting in Giessen(June 1993).

b) the continuation of the purchase and assembling of a beta spectrometer intented to characterise the energy distribution of the radiation field. A part of this action was devoted to the elaboration of a software model operating the spectrometer from PC instructions.

c) to perform the preliminary measurements on a new irradiation facility which will act as a primary radiation source of low energy X-radiation.

II.A. Objectives for the next reporting period.

The next period of the Contract is expected to enable the following actions with respect to those in progress:

- the collection of data provided by all participants will form the basis for an evaluation of uncertainties with discussion of procedures (in collaboration with others).
- the beta spectrometer and the electronic equipment for data acquisition will be tested with a radioactive source (^{207}Bi) to achieve the settings (linearity). The automatic operation of the detector will be implemented and methods of unfolding will be investigated.
- the assembly of the facility will be ended up in order to start the measurements of the conversion factors from Kair to absorbed dose to tissue depth of $7 mg/cm^2$. Angular incidence other than 0° will also be taken into account.

III. Progress achieved including publications

I - (^{147}Pm) INTERCOMPARISON

An extended area (^{147}Pm) source with an active diameter of 42 mm has been provided by CEA-Grenoble and circulated for calibration by 4 participants. The results of the inter-comparison are considered in the general part of the report. But additional data have been obtained by the laboratory for the following conditions :

Distances from the source: 15 and 20 cm
Irradiation angles: 0°, 15°, 30°, 45° and 60°

Taking into account previous conclusions related to the adequacy of the extrapolated quantity $D_t(0)$ when the thickness of the chamber window is rather large (i.e. $2.4 mg/cm^2$) the corresponding values have not been reported in TABLE 1.

	$\dot{D}t(0.02 ; \alpha)$ - $\mu Gy.s^{-1}$ -		$\dot{D}t(0.07 ; \alpha)$ - $\mu Gy.s^{-1}$	
	15 cm	20 cm	15 cm	20 cm
Angle α degree				
0°	49.8 (2.1)	12.2 (2.2)	21.9 (2.1)	4.51 (2.1)
15°		12.0 (2.2)		4.37 (2.2)
30°		11.2 (2.2)		3.87 (2.2)
45°	40.3 (2.1)	9.94 (2.2)	14.5 (2.1)	3.11 (2.2)
60°		8.04 (2.2)		2.21 (2.2)

( ) : uncertainty in % 1 σ Reference date : 01/01/93

TABLE 1: Results of measurements with ^{147}Pm CEN/G Source

The depth-dose curves (DDC) have been represented by a degree (1+3) polynomial function as given in the following expression:

$$Ln[I(x)] = Ln[I(0)] + a_1 (x) + a_3 (x)^3$$

where x: thickness of the tissue layer (in mg/cm^2)
I(x) : measured current with thickness x
I(0) : extrapolated value of current to thickness 0

The uncertainties on the values of k wi(x;wi) (TABLE 2) have been calculated in accordance with the procedure proposed in the previous report(1). The estimation of these uncertainties derives from the uncertainties on the fitted values of the ionisation currents determined for layer thicknesses ranging from the value of the entrance window thickness (wi) (0.6 or 2.4 mg/cm^2 to 10 mg/cm^2). Those uncertainties are calculated by the Fortran fitting program, and are then applied to the expressions:

$$k\ wi(2;wi) = Ic(2)/Ic(wi) = \exp[\ Ln\ Ic(2) - Ln\ Ic(wi)\ ]$$
$$k\ wi(7;wi) = Ic(7)/Ic(wi) = \exp[\ Ln\ Ic(7) - Ln\ Ic(wi)\ ]$$

where Ln Ic(2) and Ln Ic(7) are the fitted data at the thicknesses 2 mg/cm^2 and 7 mg/cm^2, respectively, and
Ln Ic(wi) is the fitted value at the thickness of the entrance window.

As a consequence, that procedure implies that the uncertainty on the dose to tissue at a specified depth, for example at 7 mg/cm^2, does not depend exclusively on the uncertainty on the measurement performed at that depth, but involves the knowledge of the whole DDC.

		K wi		K (2;wi)		K (7;wi)	
D = 20 cm	0°	1.529	(2.2)	1.075	(1.3)	0.397	(1.3)
	15°	1.534	(2.2)	1.075	(1.3)	0.393	(1.3)
	30°	1.578	(2.4)	1.080	(1.3)	0.372	(1.3)
	45°	1.681	(2.3)	1.091	(1.3)	0.342	(1.3)
	60°	1.782	(2.3)	1.102	(1.3)	0.304	(1.3)
D = 15 cm	0°	1.412	(2.2)	1.060	(1.3)	0.467	(1.3)
	45°	1.592	(2.3)	1.081	(1.3)	0.391	(1.3)

( ) : uncertainty % 1σ

TABLE 2: Fitted data involved in the estimated uncertainties

The angular conversion factors (obtained from FIGURE 1) have been compared with those of a similar source denoted (S Dos). The extrapolated values (depth = 0 mg/cm^2) are also presented because those graphs involve only normalised data. Taking into account the uncertainties on experimental results, the agreement between both sets of data is quite satisfactory.

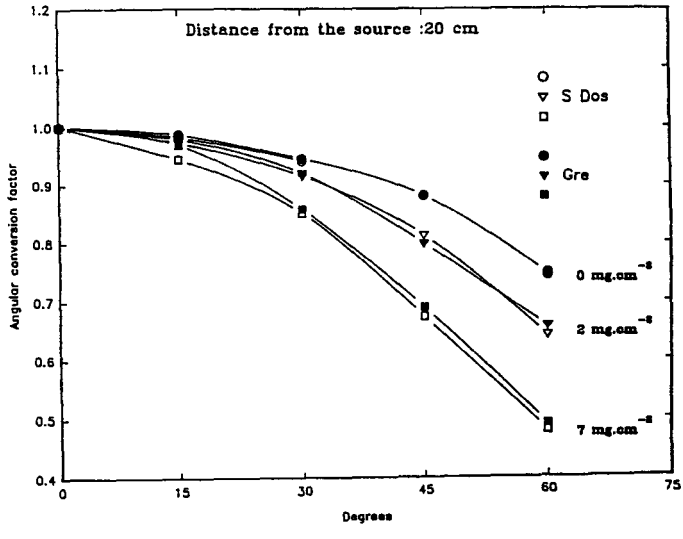

FIGURE 1: Angular conversion factors of two ^{147}Pm sources

II - BETA SPECTROMETRY

The characteristics of beta reference radiations used for calibration and type testing of dosemeters rely generally on data inferring from the depth-dose curves and the determination of the residual range. In order to get a more detailed knowledge of the radiation field, a beta spectrometer has been considered as the most efficient tool. Alterations of the initial spectrum of the radionuclide result from radiation scattering caused by the surroundings: source holders, air, flattening filters... The energy spectrum in the calibration area will suffer modifications which have to be clearly identified in terms of energy and spatial distributions.

The spectrometer consists of 2 coaxial Si detectors INTERTECHNIQUE (ref. IPT 150-300-16 and LEC 200-5000) fitted in a metallic housing. The Si thicknesses are respectively 300 µm and 5000 µm. The association of 2 detectors enables to combine the low-energy threshold of D1(IPT type) and the good efficiency of D2 (LEC type). Low energy electrons are detected by D1 only up to an energy of about 200 keV (see next paragraph). Above that energy, electrons will reach the D2 detector with a residual energy Eres = Einc - ΔE, ΔE being the energy loss in D1.

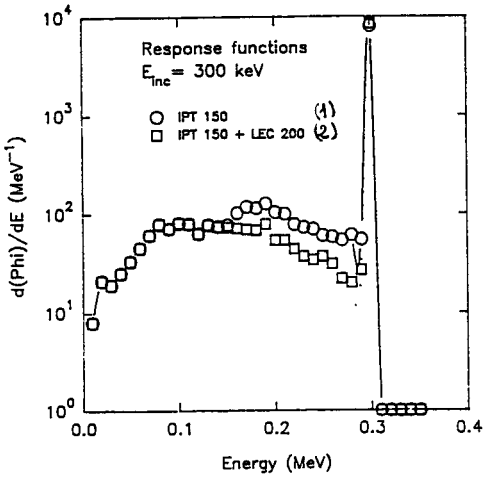

Fig. 2a

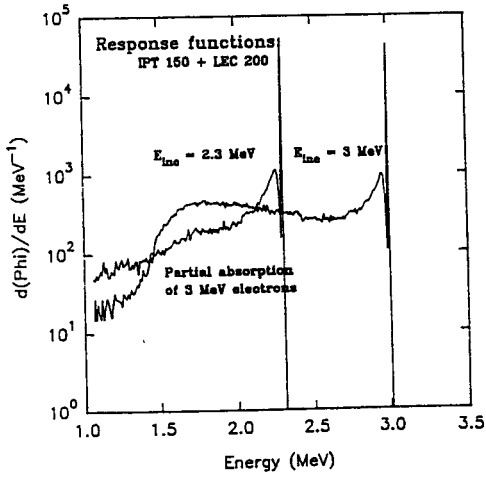

Fig. 2b

FIGURE 2: Examples of response functions

A coincidence between pulses delivered by each detector will yield an information proportional to the total energy lost in the spectrometer. The distribution of those energy losses, i.e. the response function, has been studied theoretically by means of a simulation with EGS4 code(2). In Fig. 2a, a response function for the incident energy 300 keV is represented by 2 graphs (1) and (2). Graph (1) demonstrates that an incomplete absorption of electrons in D1 is accompanied by a parasitic distribution of energy losses (compare graphs 1 and 2)

which increases with the energy of incident electrons. A similar result for E>2.3 MeV (detectors D1+D2)defines the upper limit of "safe" use of the spectrometer (no distorsion of the response function) (Fig.2b).

From those results derives an interesting feature of the response function: a total absorption peak from which a "regular" response matrix is expected (approx. triangular matrix with prominent diagonal elements). A bibliography about unfolding procedures is currently in progress (3,4).

The electronic equipment for data acquisition including pulse-shaping units and MCA have been purchased. Scanning movements of the detector in an irradiation plane are servo-controlled by orthogonal linear guides and step-motors (MICROCONTROLE). An application software to achieve a sequential movement and the associated data acquisition is being implemented on the MCA/PC.

III - CONVERSION FACTORS FOR LOW ENERGY X-RAYS

The procedures involved in the evaluation of data from measurements performed with an extrapolation chamber rely on the "quality" of the extrapolation curve. They require the availability of a high stability radiation source, and in the present case, a high performance X-ray unit. A PHILIPS generator(MG105 type) has been acquired (in collaboration with the LCIE institute). The main characteristics are as follows:

- High Voltage range: 5kV ---> 100kV
- High frequency supply: 500 Hz
- Current stability: 0.2%
- Voltage stability: 0.2%

A control of those characteristics for 2 H.V. values: 10 kV and 20 kV has been performed over an 8 hours period with an ionisation chamber. Both values belong to the range at which the X-ray facility will be operated. The maximum deviation between measured currents: 0.34% , with an uncertainty of 0.05% on each value, agrees quite well with the data claimed by the manufacturer.

REFERENCES

(1) - CEC Contract Bi7-028 - Dosimetry of beta and low-energy photon radiation using extrapolation chambers and thin solid state dosimeters. Final report (1992).

(2) - W.R.Nelson,H.Hirayama,D.W.O.Rogers - EGS4 Code System, SLAC-235, Stanford Linear Accelerator (1985).

(3) - F.Hajnal - Beta radiation measurement: Evaluation of scintillation detector measurements. Proceedings of the International Beta Dosimetry Symposium. Washington D.C. February 15-18, 1983.

(4) - T.Novotny - Determination of a photon response matrix simultaneous measurement of neutron and photon spectra in mixed fields. Radiation Protection Dosimetry Vol.44 n° 1/4 pp 93-96 (1992).

Head of project 3: Mr T M Francis

II. Objectives for the reporting period

1. Participation in intercomparison exercises conducted among the collaborating laboratories with a view to standardising procedures and measurements.
2. Characterisation of beta radiation field from $^{90}Sr/^{90}Y$ foil source in terms of ISO specifications[1] for a series 2 secondary standard at distances that were not covered under the previous contract.
3. Design and fabrication of shields for the study of photon spectra (bremsstrahlung and characteristic x-rays) from the above mentioned source.
4. Characterisation of photon spectra and provision of data obtained as an input to computational models that are being developed as part this programme.

II.A. Objectives for the next reporting period

During the next period of the contract, measurements for estimating the photon spectra from this source (both qualitatively and quantitatively) as well as their modification due to absorber thickness will continue. Measurements of beta spectra at various calibration distances and their modification due to factors such as air path, external absorbers and source orientation with respect to the detector plane are also planned. An attempt will be made to establish relationships between measured beta dose rates and beta spectra in conjunction with participants who are developing computational models

III. Progress achieved including publications

As a part of the agreed procedure for standardising the extrapolation chamber measurements among the participating laboratories, an intercomparison of measurement was carried out using an extended area ^{147}Pm foil source. Measurements of $H'(0.07;\alpha)$ at 0.15 and 0.2 m with α at 0° and 45° and depth dose at these distances and orientations were undertaken. The preliminary results show good agreement among the participants and are reported in some detail under "Summary of Project Global Objectives and Achievements".

The work on the characterisation of the beta radiation field from the extended area $^{90}Sr/^{90}Y$ source (previously acquired[2]) continued. Measurements of dose rates, depth-dose at different angles and distances not covered (0.15 and 0.4 m) under the previous contract and the measurement of residual maximum beta particle energy, E_{res}, at these distances were completed. The details of the source construction and the design of the holder as well as the methodology used for these measurements were given previously[2]. Table 1 gives all the results obtained so far. For completeness some of the results obtained and reported under the previous contract[2] are also included in the table. The results show that at this range of distances, variation in dose rates with distance (approximately an order of magnitude from 0.15 to 0.4 m is more or less consistent with that predicted by the inverse square law. This gives a certain degree of flexibility in the choice of dose rates by the variation of distance as may be required for some experimental applications. As the source to detector distance is increased the maximum value of "build up" and its dependence with orientation gradually decreases. As would be expected, the value of

E_{res} decreases with distance and at 0.4 m it is just below the minimum specified[1] value.

The work on estimating the mean energy of and dose rate due to the bremsstrahlung and characteristic x-rays that generally accompany beta rays from a practical source such as the one in use here has commenced. For this purpose a special holder with PMMA (polymethyl methacrylate) was made with an absorber thickness just sufficient to stop the most energetic beta radiation from the source. Figure 1 shows schematically the construction of this holder. With the source placed in this holder the radiation emerging from the surface of the holder will be a photon spectrum (totally free from beta radiation) composed of bremsstrahlung and characteristic x-rays from the source itself, that generated in its holder and the external shielding used. A GM counter (Mini Instruments GM Probe Type E) in conjunction with a Mini Instrument Scaler ratemeter Type 6-90 was used to measure the count rate due to these photons. The count rate as a function of absorber (lead) thickness was measured at distances of interest. The dose equivalent rate due to the photon spectra at these distances from the special source holder (with source placed in it) was measured with an ion chamber (Eberline Ion Chamber, Model RO-10). Simple analyses based on attenuation coefficients for photons in lead of the measured transmission curves indicate that the photon spectrum consists of two distinct components, one of low energy (≈85 keV) and the other of moderately high energy (700-800 keV). This is shown by the rapid fall of the transmission curve initially which is followed by a tail that decreases relatively slowly with absorber thickness. Figure 2 shows measured transmission curves at three source to detector distances (0.2, 0.3, 0.4 m). Transmission curves obtained at these distances have almost an identical shape with the result they are not distinguishable from each other. This indicates that the composite photon spectrum has not undergone significant change in shape with distance at least in this range of distances. The last column in Table 1 gives $H'(0.07;0°)$ due to photons (bremsstrahlung and characteristic x rays) from the source at three distances as measured by a typical monitoring instrument (Eberline Ion Chamber, Model RO-10). These are less than 0.05% of the total $H'(0.07;0°)$ at these distances.

References

1. ISO. Reference Beta Radiations for Calibrating Dosemeters and Doserate Meters and for Determining their Response as a Function of Beta Radiation Energy. ISO 6980. (Geneva: International Organisation for Standardisation) (1984).

2. Final Report for Contract No. Bi7-028, 1992.

Figure 1: Schematic diagram of the special holder used for the studies of photon spectra from a $^{90}Sr/^{90}Y$ foil source.

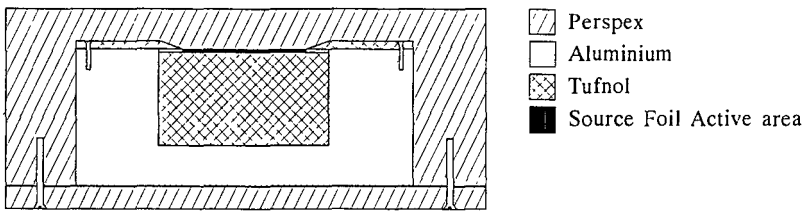

Figure 2: Transmission (normalised to unity at zero thickness) of photon spectra from a $^{90}Sr/^{90}Y$ foil source through lead at three source to detector distances (0.2, 0.3 and 0.4 m).

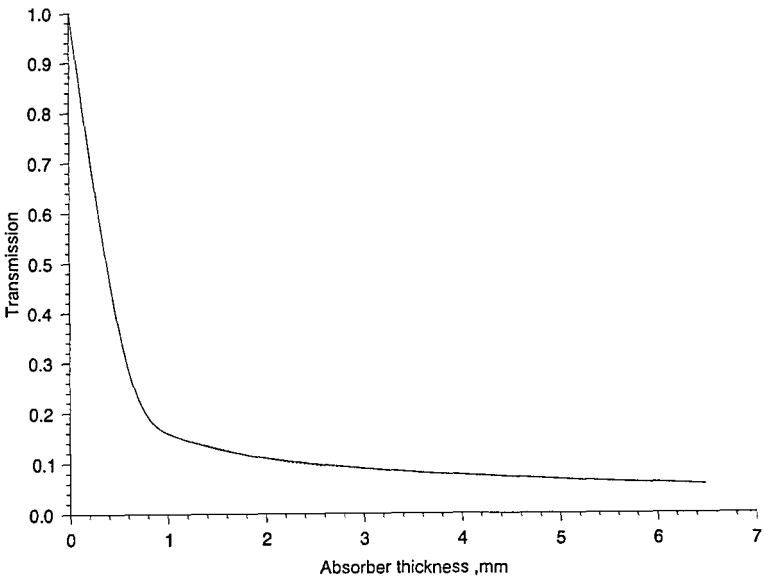

Table 1: Directional dose equivalent rates, $\dot{H}'(0.07;0°)$ for four distances and their variation with orientation (normalised at 0°), residual maximum beta particle energy, E_{res} and directional dose equivalent rate (only due to photons), $\dot{H}'(0.07;0°)$ obtained for a $^{90}Sr/^{90}Y$ foil source of nominal activity of 370 MBq.

Distance m	Tissue depth,d kg/m²	$\dot{H}'(d;0°)$ mSv/h	$\dot{H}'(d;\alpha)/\dot{H}'(d;0°)$					Residual Emax MeV	$\dot{H}'(0.07;0°)$ photons only mSv/h
			α=0°	α=15°	α=30°	α=45°	α=60°		
0.15	0.07	421	1.00	1.02	1.07	1.13	1.15	1.98	--
0.2	0.07	239	1.00	1.02	1.06	1.12	1.12	1.95	0.08
0.3	0.07	104	1.00	1.02	1.07	1.09	1.08	1.86	0.04
0.4	0.07	56	1.00	1.03	1.05	1.10	1.07	1.76	0.02

Head og project 4: Dr. Herbaut

II. Objectives for the reporting period

- Establishment of computer - controlled automated extrapolation chamber measurement set-up (chamber PTW model 23392).
- Control of the reliability of the extrapolation chamber for measuring absorbed dose rate in tissue; the laboratory has participated in comparative measurements of dose rate from an extended area ^{204}Tl source, organised by the coordinator of the project.
- Determination of conversion factors $G'(0.07,\alpha)$ for different values of α for point sources (^{147}Pm, ^{204}Tl, $^{90}Sr + ^{90}Y$) belonging to the Büchler secondary standard using carbon loaded LiF dosemeters.

II.A Objectives for the next reporting period

1 - Determination of the influence of the diameter of the collecting electrode (PTW model 23391 chamber), using the ^{204}Tl extended source applied for the intercomparison. Study of the influence of the holder of the source (French and NRPB holder).

2 - Control of reliability of extrapolation chambers for the measurement of absorbed dose rate to tissue; the laboratory will participate in comparative measurements of dose rate from a 42 mm diameter ^{147}Pm source organised by the coordinator of the project. A PTW model 23392 chamber will be used and the results compared with those previously obtained with the PTW model 23391 extrapolation chamber.

3 - Determination of the residual maximum beta energy of the spectrum at different calibration distances for the beta sources used at CEA/Grenoble.

4 - The study of the characteristics of carbon loaded LiF dosemeters will be continued.

III. Progress achieved including publications

1. Automation of the extrapolation chamber

In order to override the problems due to the weak activity of the beta sources and to decrease the risk of experimenter irradiation, a new extrapolation chamber (PTW model 23392) has been automated. Its main characteristics are:

- entrance window

- material: mylar
- thickness: 0.75 $mg.cm^{-2}$

- collecting electrode

- material: perspex
- diameter: 30 mm

- field strength 10 $V.mm^{-1}$

The measurements are very time consuming and a fully computerised automated procedure is essential.

As with the 23391 PTW device, this detector uses an acquisition program that includes knowledge of ambient parameters.

2. Intercomparison of extended area ^{204}Tl source /1/

An extended area ^{204}Tl source (active diameter 42 mm) with the standard french holder was used for this intercomparison.

The laboratory at CEA/Grenoble has contributed to this exercise with the model 23391 PTW chamber. For determination of tissue dose rate $\dot{D}_t(0.07,\alpha°)$ the chamber was equipped with a 8.5 mg.cm^{-2} kapton entrance window, equivalent to 7.8 mg.cm^{-2} of tissue (W.G. CROSS gives a relative attenuation of 0.92 for mylar wich has nearly the same density as kapton). We have not applied any correction factor between 7.8 and 7.0 mg.cm^{-2}.
The measurements were performed at three distances d (15, 20, 30 cm) from the source and at irradiation angles α = 0° and 45°.

Table 1 presents the results of $\dot{D}_t(0.07,\alpha°)$.

$\dot{D}_t(0.07,\alpha°)$ in mGy.h^{-1}					
d = 15 cm		d = 20 cm		d = 30 cm	
α = 0°	α = 45°	α = 0°	α = 45°	α = 0°	α = 45°
411.6 ± 1.7 %	383.3 ± 1.7 %	218.0 ± 1.7 %	200.0 ± 1.7 %	85.1 ± 1.6 %	75.2 ± 1.6 %

Table 1 - Measured tissue dose rates $\dot{D}_t(0.07,\alpha°)$.Estimated uncertainties (± 1 S.D) are indicated below each dose rate value (Reference date 01.01.1991).

Our results agree well with the data provided by the other participants for 20 and 30 cm /1/. There may be a problem of calibration of the length sensor for the shorter distance.
For the depth dose curves, the thickness of this entrance window was 4.5 mg.cm^{-2} of kapton (equivalent to 4.14 mg.cm^{-2} of tissue).
The measurements were performed at three distances d (15, 20, 30 cm) from the source and at irradiation angles α = 0° an 45°.
Figure 1 shows an example of depth dose curves for 20 cm.

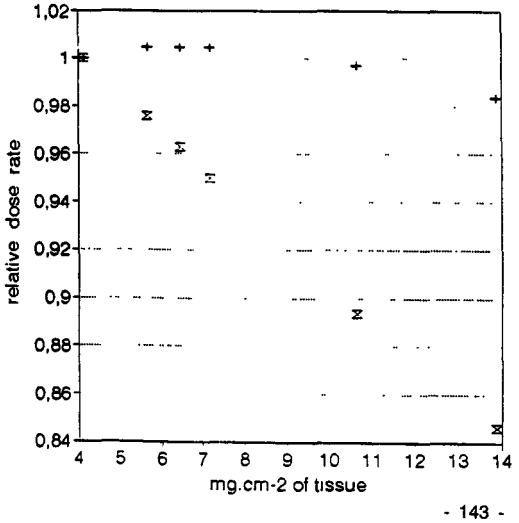

Figure 1 - Results of measurements of depth dose profiles for the ^{204}Tl source obtained at distance of 20 cm from the source and for irradiation angles α.

\+ α = 0°
x α = 45 °

All data are normalised to depth : 4.1 mg.cm^{-2}

3. Thermoluminescent dosemeters

During recent years, the importance of beta dosimetry has increased, at least in part, due to an awareness of the beta problem in nuclear plant /2/.

Therefore, we have investigated the properties of carbon loaded LiF détectors /3/ manufactured by N.E. Technology (Vinten) /3/ and their applicability for the determination at different irradiation angles of conversion factors $G(0.07,\alpha°)$

$$G(0.07,\alpha°) = \frac{\mathring{D}_t(0.07,\alpha°)}{\mathring{D}_t(0.07,\alpha°=0°)}$$

The detectors were positionned on a perspex 2 cm thick slab which can be tilted, and were then covered by a thickness of mylar 3.8 $mg.cm^{-2}$.

The results are presented in Table 2 and on Figures 2 and 3.

Radionuclide	R_β	Standard deviation
$^{90}Sr + ^{90}Y$	1.00	
^{204}Tl	0.93	23 %
^{147}Pm	0.99	10 %

Table 2 - The ^{147}Pm, ^{204}Tl, and $^{90}Sr + ^{90}Y$ sources are point sources from the Büchler secondary standard. R_β is the response per unit $\mathring{D}_t(0.07,\alpha = 0°)$, normalised to that of $^{90}Sr + ^{90}Y$.

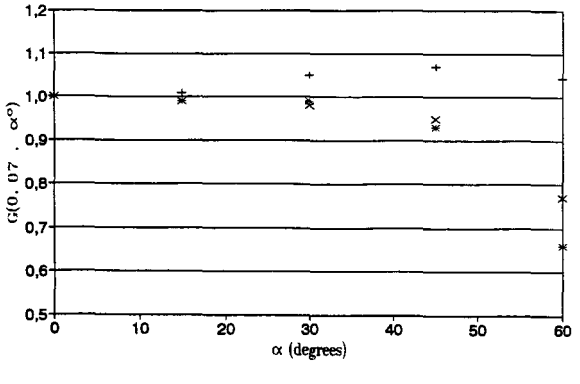

Figure 2 presents the value of conversion factors $G(0.07,\alpha°)$ as a function of the angle α for $^{90}Sr + ^{90}Y$ point source without filter at 30 cm distance.

\+ extrapolation chamber (model PTW 23391)

* 0,13 mm LiF dosemeters (covered with 7 $mg.cm^{-2}$ mylar) /1/

x carbon loaded LiF dosemeters (covered with 3.8 $mg.cm^{-2}$ mylar).

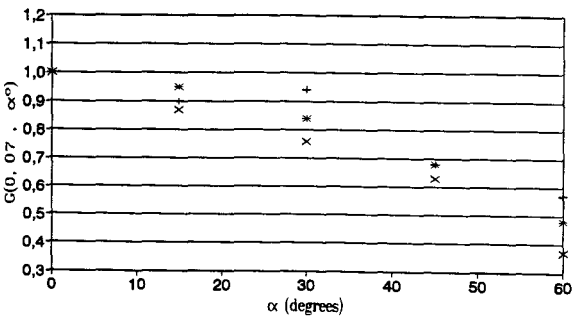

Figure 3 presents the value of conversion factors $G(0.07,\alpha°)$ as a function of the angle α for ^{204}Tl and ^{147}Pm point sources (Büchler) with filter.

\+ ^{204}Tl } 0,13 LiF dosemeters
x ^{147}Pm } (covered with 7 mg.cm^{-2} mylar) /1/)

* ^{204}Tl Carbon loaded LiF dosemeters (covered with 3.8 mg.cm^{-2} mylar)

The small difference in the results obtained using the 0,13 LiF dosemeter /1/ and the carbon-loaded LiF detector was unexpected. This result puts into question the measurements obtained with the carbon-loaded LiF detector ; further measurements will be performed.

^{147}Pm sensivity has also been determined in the course of the contract BI 7-021 with Dr CHARLES (Nuclear Electric) concerning hot particle dosimetry (^{60}Co spherical sample). In this case, carbon-loaded LiF dosimeters were covered with a 7 mg.cm^{-2} mylar foil (Table 3).

Radionuclide	R_β	Standard deviation
$^{90}Sr + ^{90}Y$	1.00	
^{147}Pm	0.50	12 %

Table 3 - ^{147}Pm and $^{90}Sr + ^{90}Y$ sources are point sources from the Büchler secondary standard. R_β is the response per unit $\mathring{D}_t(0.07,\alpha = 0)$ normalised to that of $^{90}Sr + ^{90}Y$

Comparison of Table 2 and Table 3 shows the importance of the thickness of the covering material for low-energy weakly penetrating radiations.

4. References

/1/ Final Report of CEC Contract BI7-028 (1992)

/2/ CHRISTENSEN, P., JULIUS, H.W., MARSHALL, T.O.
Implication of New CEC Recommandations for Individual Monitoring for External Radiations Doses to the Skin and the Extremities
Rad. Prot. Dosim. 39. (1/3) 91-94 (1991)

/3/ BURGKARDT, B. KLIPFEL, A.
Dosimetric Properties of Carbon loaded LiF Detectors for Beta Photon Extremity Dosimetry
Rad. Prot. Dosim. 33. (1/4) - 275 - 278 (1990)

Head of project 5: Dr. C.A. Perks

II. Objectives for the reporting period

(i) To develop computer programs for determining source terms for beta-particle sources.

(ii) To model the beta particle source holder which is used by NRPB in their calibration laboratory using a Monte Carlo program based on the EGS4 system.

IIA. Objectives for the next reporting period

(i) To calculate the dose under the extrapolation chamber window of the chamber used at NRPB.

(ii) To validate the computer models by comparing the calculated results with measurements made at the NRPB.

(iii) To model factors affecting the source spectrum such as the inhomogeneity and holder design.

(iv) To model the energy and angular distributions of relevant sources.

(v) To study the effects of beam flattening filters on the radiation field and to optimise filters.

III. Progress achieved including publications

BETA ENERGY SPECTRA

A computer program which generates beta spectra cumulative probability distribution functions and frequency distribution functions has been developed. Spectra generated using this program have been compared with tabulated graphs of beta spectra and found to match them very closely. This program will be used to generate beta particle spectra in the modelling of beta sources.

A Monte Carlo program has been developed for EGS4 which is able to sample spectra from cumulative probability distribution functions.

BETA SOURCES

A method for storing the spectra produced by beta sources has been developed. This was needed to be able to perform rapid sampling of spectra. For each emitted beta particle or photon, the energy angle, and co-ordinates of the point of emission are required. For a given source this uses a considerable amount of computer memory. This has been minimised by developing a method for recording data in single bytes.

The stored spectra will be used to start beta histories for different dosemeters without following the production of particles through the source holder. This will save computer running time and enables variance reduction due to the correlation of histories, if two runs are carried out using the same source spectrum.

Two sources have been modelled to date, a simple cylindrical source holder and a model of the cuboid beta source holder used by the NRPB. Considerable care has been taken to determine the exact composition of the structural materials of the source. A comparison with measured spectrum will be made.

Head of project 6: Prof. Gasiot

II. Objectives for the reporting period

The overall objectives of the actual contract are to study, develop and apply the TLD laser heating technique for dosimetry of weakly penetrating radiations. The laser heating technique offers the possibility of using detectors with a thin effective thickness which is important for dosimetry of weakly penetrating radiations. This technique, operating with short reading times, is valuable for dosimetry applications where many measurements are involved and offers the possibility of replacing the actual radiographic film technique [1-5].

The objectives for the reporting period concentrate on:

-Development of appropriate dosemeters for application of the TLD laser-heating technique to personal dosimetry. The development work is mainly concerned with $CaSO_4$ and Al_2O_3 TL materials. Important dosimetric characteristics of the dosemeters for application for individual monitoring will be investigated.

- Development and testing of thin film detectors for "hot particle" dosimetry to map the dose distribution originating from the beta radiation near the surface of the radioactive particle.

- Determination of dosimetric characteristics of thin TLD detectors based on laser heating for the measurement of H' (d;a°) in beta radiation fields.

II.A. Objectives for the next reporting period

The objectives for the next reporting period are currently under investigation in collaboration with RISØ National Laboratory. They are concerned with the development of thin film dosemeters based on activated $CaSO_4$ and Al_2O_3 suited for laser heating for characterising beta radiation fields in terms of H' (d;a°). Dosemeter responses of small as well as large-size TLD plates and pellets will be measured for different beta energies and irradiation angles. The performance of the laser reader will be compared to that of a conventional reader as initiated in the previous contract.

III. Progress achieved including publications

III.1. Development of dosemeters adapted to laser heating and to beta dosimetry.

The work is related to the conception, the simulation and finally the test of dosemeters adapted to the new TLD reader and to radiation dosimetry of low energy radiations. Dosemeters have been developed to measure doses, in particular, from beta rays ($^{90}Sr/^{90}Y$, ^{147}Pm, ^{204}Tl). Improvements of the dosemeters have been achieved by varying the kind of support and the TL material. In this initial work we have been concerned by material and dosemeter optimisation, measurements have been achieved using a conventional reader. Dosimetric characteristic changes have been studied as a function of dopant concentration, annealing temperature and grain size. A reading procedure allowing good reproducibility has been developed for each TLD material. For $CaSO_4$:Dy, seven TL peaks are observed at temperatures ranging from 80-100°C for peak one, to 580-620°C for peak seven. Peak three, at about 220-240°C, is called the "dosimetric peak". The recommended procedure is: irradiation at room temperature, one hour waiting to eliminate fading, preannealing to eliminate low temperature peaks 1 and 2, reading. These studies are very important for achieving appropriate dosimetry [6]. Results show that the relative peak hights of peak 1, 2 and 3 are strongly dependent upon dopant concentration, annealing temperature, grain size etc. With an irradiation dose of 4 mGy from a $^{90}Sr/^{90}Y$ source, the strongest TL signal is observed using $CaSO_4$:0.1% Dy pellets deposited on aluminium (10mm diameter and 1mm thick), an annealing temperature of 500°C and a grain size between 25 and 50 µm (fig. 1). However, fading, reproducibility and homogeneity tests indicate that the best annealing

temperature is 700°C, as TL intensity is not the only parameter to be considered. The experimental lower dose limit is then 60 μGy [7].

III.2. Testing the reader.

The configuration of the new reader allows to control very precisely five parameters: the laser power, the laser beam diameter, which can vary between 0.3 and 2 mm, the spacing between heated points, the heating time (the time during which the laser beam hits the dosemeter) and the diameter of the heated spot [8].

The initial activity has been concentrated on dosemeter preparation and test. To show the potential of the laser TLD technique for personal dosimetry, we have performed some test experiments for a 2D dosimetry, using plates of $CaSO_4$:Dy 30x40 mm^2 large, an X ray irradiation of mean energy E=100keV and filters of 5mm of Cu and 1mm of Sn. The dosemeters were covered with a plastic foil, as it is normally used in personal dosimetry, with metal filters of different thickness placed inside. The irradiation dose ranged from 0.25 to 4 mSv. Dose mapping allowed clearly to distinguish the areas covered by the metal filters down to a dose of 0.1 mSv (fig. 2). These first results indicate that TLD films, read out after irradiation by a laser heating scanning, may be used to replace the usual radiographic films.

III.3. "Hot particle dosimetry".

The most relevant point in our work has been to study the feasability of dose mapping in the mm scale for hot particle dosimetry using the laser TLD system or OSL (Optically Stimulated Luminescence). These methods are attractive compared to other possible techniques used to evaluate the dose, like extrapolation chambers (limited spatial resolution) or radiochromic dye films, which have the disadvantage of limited dynamic range and very long exposure times due to low sensitivity.

The "hot particle" was a ^{60}Co source prepared by Dr. Charles (Nuclear Electric). We received the source from Dr. Herbaut (CEN Grenoble) who had performed measurements of dose rates using an extrapolation chamber.

For the TL measurements our detector was $CaSO_4$:Dy, grain size 25-50μm, on kapton, 50μm thick. For OSL, the detector was rare earth activated MgS, about 20μm thick, deposited on kapton. For the irradiation, we placed the source, positioned in a plastic foil, in contact with the detector sticked on a plexiglas plate. We assume that the gamma radiation emitted by the source and deposited in the detector can be neglected compared with the beta rays. To check the linearity of the dose response of the detector, pellets of $CaSO_4$:Dy were irradiated for various times: 5, 10, 15, 30 and 60 min. and read with a conventional reader. The results confirmed a linear relationship. Then we irradiated the $CaSO_4$:Dy on kapton and maped the dose distribution due to the beta rays. The reading conditions were: spacing between reading points: 0.4 mm; diameter of the laser beam: 0.3 mm. The profile of the dose distribution of the maximum dose region shows that there is only a slight contribution from the gamma rays (fig. 3). The dose profile of the beta peak region in isodose fits a gaussian distribution. At 100% of the maximum of the dose, a flat dose level over a diameter of 3 mm is seen (fig. 4).

To picture the spatial dose distribution we also performed OSL measurements. In this case, the irradiated luminescent material is stimulated by infrared light. Electrons leaving traps recombine, producing an emission of visible light. Only a few milliseconds of stimulation are necessary and a bright visible light is observed. This represents a real advantage for dose evaluation over radiochromic dye films [9].

We performed calibration of the O.M.A. (Optical Multichannel Analyser) ensuring that there was no deformation of the picture. The dose profile observed after irradiation shows a gaussian distribution and the maximum of the observed OSL intensity is proportional to the duration of irradiation (below the saturation of the detector) (fig. 5).

To estimate the average beta dose deposited in the material we integrated the area under the gaussian OSL signal and irradiated the same detector area with a calibrated 50 keV X-ray source. Assuming that in our configuration, the beta dose is deposited uniformely in the material, that the OSL signal is due solely to collisions of the electrons within the detector, and

that the light intensity is proportional to the deposited dose, we estimated the dose to be about 0.6 Gy/hour. Of course, the next step will be to perform Monte Carlo calculations using the present configuration in order to confirm the experimental results. This will be done in collaboration with Dr. Patau from the University of Toulouse.

III.4. Dosimetric characteristics of thin TLD detectors for beta dosimetry.

A programme for studying the dosimetric characteristics of thin TLD detectors based on laser heating for beta dosimetry has been setup in collaboration with RISØ National Laboratory who will take care of the irradiation of the detectors at different beta energies and angles of the incident radiation. This work has started and will continue during the next reporting period.

Publications and bibliography

1. Gasiot, J., Fillard, J. P. and Bräunlich, P. Laser Heating in Thermoluminescent Dosimetry. J. Appl. Phys. 53, 5200-5208 (1982).
2. Bräunlich, P., Tetzlaff, W., Gasiot, J. and Jones, S. C. Development of a Laser Heating TLD Reader. In: Proc. Int. Beta Dosimetry Symp. Washington DC. February 15-18, 293 -305 (1983).
3. Christensen, P. and Prokic, M. S. Energy and Angular Response of TL Dosemeters for beta ray Dosimetry. Radiat. Prot. Dosim. 17, 83-87 (1986).
4. Christensen, P., Herbaut, Y. and Marshall, T. O. Personal Monitoring for external sources of beta and low Energy Photon Radiations. Radiat. Prot. Dosim. 18, 241-260 (1987).
5. Servière, H., Vignolo, C. Prévost, H. and Gasiot, J. Laser Heating of TL dosemeters: Application to beta Dose Mapping. Radiat. Prot. Dosim. 39, 145-148 (1991).
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8. Gasiot, J. CEC Contract N° Bi7-028 Report August 1992.
9. Missous O., Loup F. Fesquet J., Prévost, H., and Gasiot, J. Optically stimulated luminescence (OSL) of rare earths doped phosphors. Eur. J. Solid State Inorg. Chem. 28/S, 163-166 (1991).

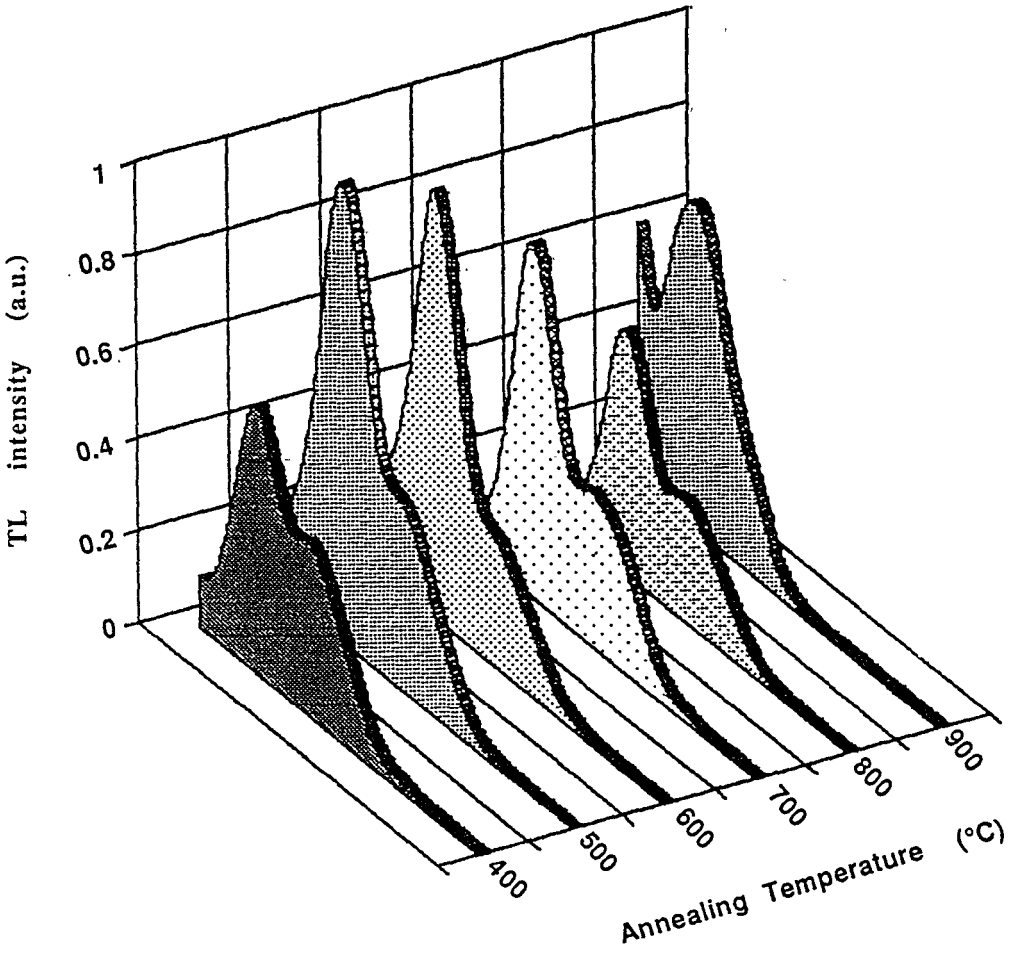


Fig. 1 : Glow curves of CaSO4: 0.1% Dy as a function of annealing temperature
(dose : 4 mGy; source : $^{90}Sr/^{90}Y$).

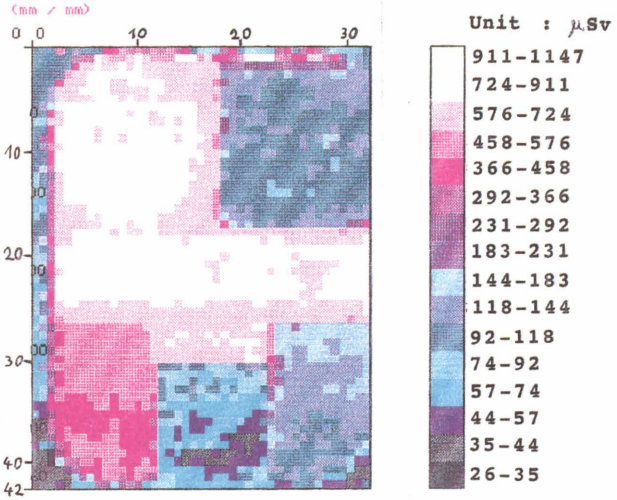


Fig. 2 : 2D dosimetry of a $CaSO_4$: 0.1% Dy film in a plastic badge with filters placed inside

(source: X ray, E=100 keV; dose: 0.75 mSv; filters: Cu=5 mm; Sn=1 mm).

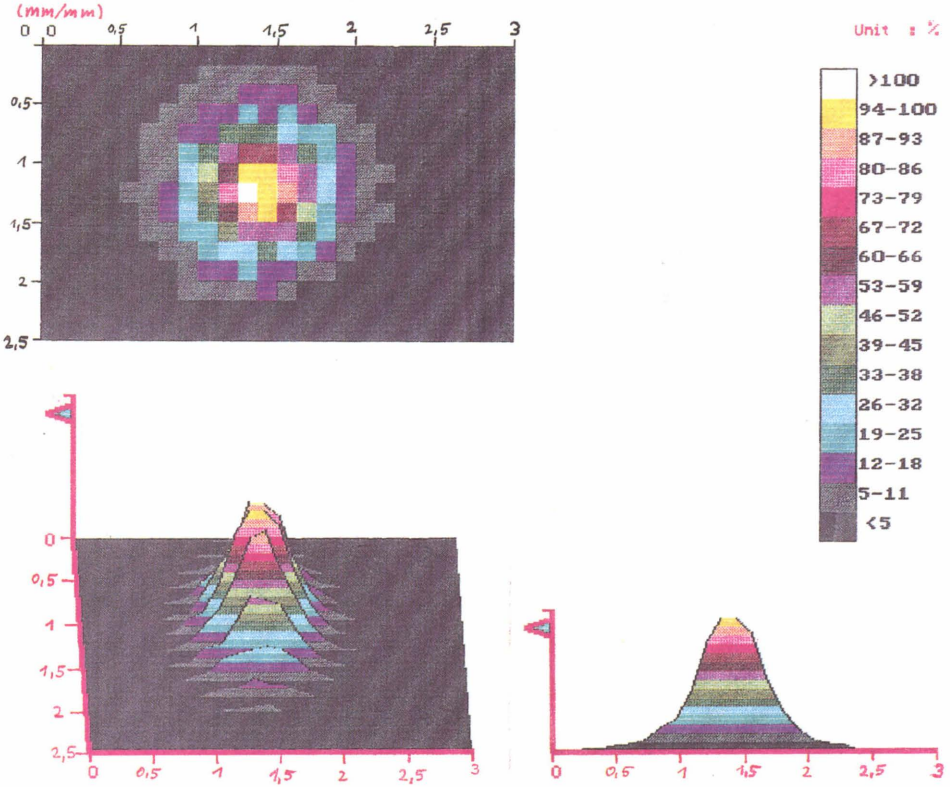


Fig. 3 : Dose distribution of a "hot particle" with laser heating TLD technique (^{60}Co source; detector: $CaSO_4$: 0.1% Dy on 50 µm kapton).

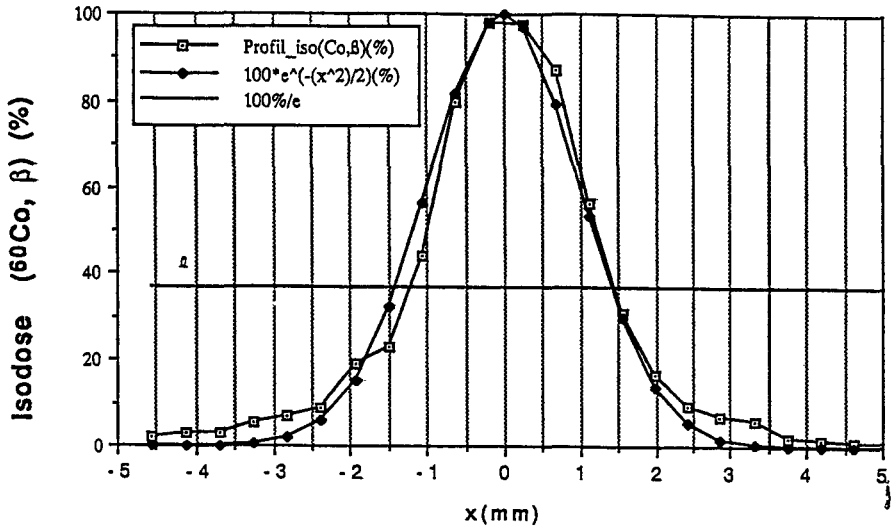


Fig. 4 : Isodose Profile of the beta peak region (experimental curve and calculated gaussian distribution) (^{60}Co source; detector: CaSO4: 0.1% Dy film on 50µm kapton).

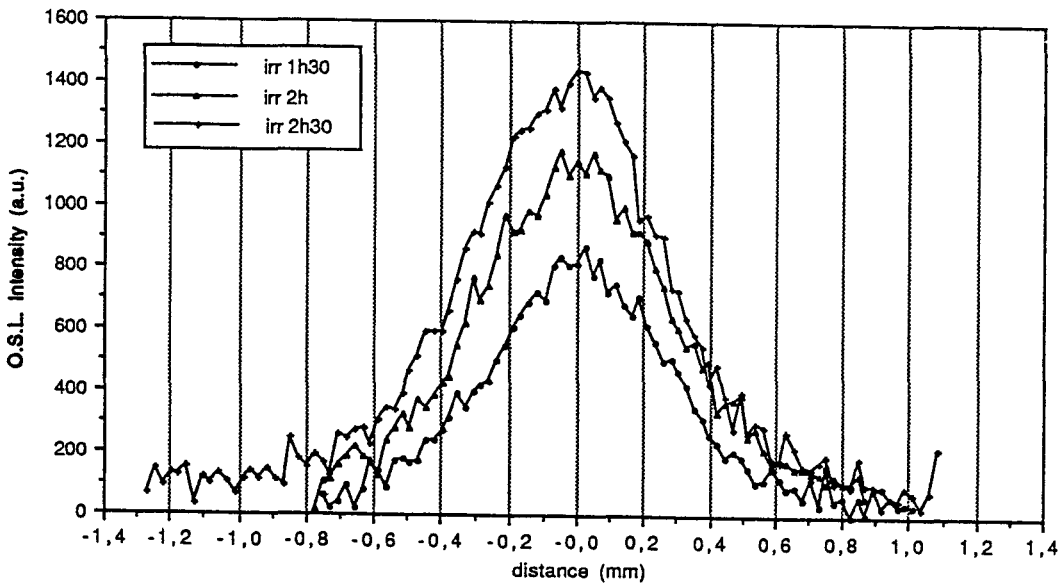


Fig. 5 : Beta Dose profiles deposited by a ^{60}Co source on an OSL detector after irradiation.

Head of project 7: Prof. Dr. Scharmann

II. Objectives for the reporting period

Test of window materials for an optimized TSEE dosemeter badge for individual monitoring in mixed beta/gamma radiation fields. Parameters studied:
- Dependence of the TSEE response on photon energy and composition of cover materials.
- Response of BeO thin films to beta radiation.

II.A. Objectives for the next reporting period

- Analysis of the reliability of BeO thin film detectors produced by the Battelle Institute in Frankfurt and the Staatliches Materialprüfungsamt in Dortmund (already in progress).
- Analysis of the surface composition of TSEE detectors from different origin by secondary ion mass spectroscopy (SIMS).
- Construction of a dosemeter badge with Lutamer window and test in photon and beta radiation fields.
- Characterization of ^{14}C and ^{63}Ni low-energy beta radiation fields.

III. Progress achieved including publications

1. Dependence of the TSEE response on photon energy and composition of cover materials

The TSEE sensitivity to X-rays is strongly dependent on the atomic number Z of cover materials above the exoelectron emitting layer. At higher Z it is almost proportional to the mass energy transfer coefficient of the covers (Fig. 1). From Fig. 1 can be concluded that electrons originating from photon interactions in the cover material give the major contribution to the occupation of TSEE centres in BeO thin films. TSEE detectors approach the behaviour of Bragg-Gray detectors. The data in Fig. 1 refer to cover foils consisting of Be, graphite, plastics, Al, Cu, Ag and Ta. In order to establish secondary electron equilibrium the foils were slightly thicker than the range of photoelectrons, but as thin as possible to avoid photon attenuation.

For the construction of an optimized badge the dependence of the TSEE response on photon energy was determined for four different 7 $mg \cdot cm^{-2}$ plastic covers, whose effective atomic number Z_{eff} ranged from 6.7 - 9.7. In a first series of measurements carried out with ^{60}Co, monoenergetic X-ray fluorescence and heavily filtered X-ray sources of the CEA institute in Fontenay-aux-Roses the detectors were irradiated with normal radiation incidence on a 30 x 30 x 15 cm PMMA phantom. All exposures took place at constant air kerma. The TSEE response data in Fig. 2a are normalized to constant H(0.07) with the conversion coefficients for the PMMA phantom (details are described in (1)). The heights of the response maxima at photon energies of 30 - 40 keV differ by almost the same factors as the mass energy transfer coefficient of the foils. Similar results were obtained for irradiations free in air and normalization of the TSEE response to constant air kerma (Fig. 2b). These measurements were carried out with photon sources of the GSF, Neuherberg.

The best energy independence was achieved with detectors covered by Lutamer. The response varied between 0.77 and 1.00 in the photon energy range from 15 keV - 1.25 MeV, corresponding to deviations of ± 13 % referred to a mean response value. Lutamer is a black intrinsically conducting polypyrrole material, recently developed by BASF. It is mechanically and chemically stable and prevents optical fading. Because of its electric conductivity disturbing charging effects at the TSEE active BeO layer of the detectors are avoided.

2. Response of BeO thin films to beta radiation

The characteristics of BeO thin films for beta radiation detection have already previously been described. However, as yet no ideal cover material was available. For this reason the above mentioned cover foils, and particulary Lutamer were also tested in beta radiation fields. Lutamer windows provide an almost energy independent response to beta radiation (Table 1). The PETP foil was 10 % thicker than assumed and declared by the manufactorer. This caused a lower sensitivity to ^{147}Pm in Table 1. The sensitivity to $^{90}Sr/^{90}Y$ was the same as to ^{90}Co for all 4 plastic foils (Table 2). The dependence of the response to $^{90}Sr/^{90}Y$ on the thickness of Lutamer covers is in accordance with the transmission factors for tissue (Fig. 3). Fig. 4 shows the angular dependence of the response to $^{90}Sr/^{90}Y$ in comparison to the conversion factors $F(0.07,\varphi)$. The deviation at angles of 75° can be avoided if flat BeO thin film detectros are used instead of standard detectors. On the basis of these results Lutamer will be used as window for an optimized badge construction.

Publication:

(1) W. Kriegseis, K. Rauber, A. Scharmann, K.-H. Ritzenhoff, J.L. Chartier, D. Cutarella, C. Itié, M. Petel, *Dependence of the TSEE response of BeO thin films on photon energy and composition of cover materials*, Radiat. Prot. Dosim. **47**, 143-146 (1993).

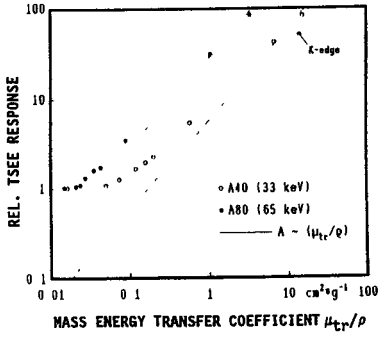


Figure 1. Relationship between TSEE response and mass energy transfer coefficient μ_{tr}/ρ of cover materials on BeO thin film detectors for constant exposures to heavily filtered A40 and A80 X-rays.

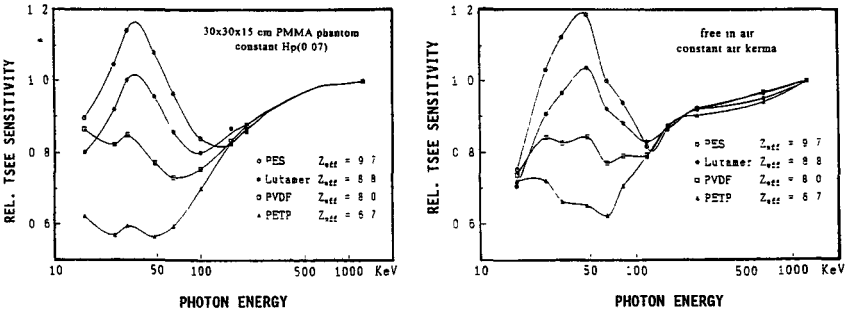


Figure 2. TSEE response to photons of BeO thin film detectors covered by 7 mg•cm^{-2} polyethersulfone (PES), Lutamer, polyvinylidenfluoride (PVDF) or polyethylene terephthalate (PETP). The TSEE data are mean values from 9 detectors. *Left:* Irradiations on a 30 x 30 x 15 cm PMMA phantom, TSEE response mormalized to constant H(0.07). *Right:* Irradiations free in air normalized to constant air kerma.

nuclide	mean energy (keV)	TSEE response			
		PETP	PVDF	Lutamer	PES
$^{90}Sr/^{90}Y$	800	1.02	1.04	1.00	1.02
^{204}Tl	240	0.95	1.00	0.96	0.98
^{147}Pm	60	0.78	0.93	0.98	0.93

Table 1. Dependence of the response of BeO thin films to beta radiation on cover materials (mean values from 8 detectors) for constant H'(0.07,0°). Irradiations with Buchler sources at GSF, Neuherberg. Detectors on 13 x 13 x 1 cm PMMA slab during exposures.

PETP	PVDF	Lutamer	PES
1.00	1.06	1.02	1.04

Table 2. Response to $^{90}Sr/^{90}Y$ relative to ^{60}Co (mean values of 9 detectors) for constant H'(0.07,0°). Exposures to ^{60}Co on 30 x 30 x 10 cm PMMA phantom at CEA, Fontenay-aux-Roses. Exposures to $^{90}Sr/^{90}Y$ on 30 x 30 x 3 cm PMMA slab.

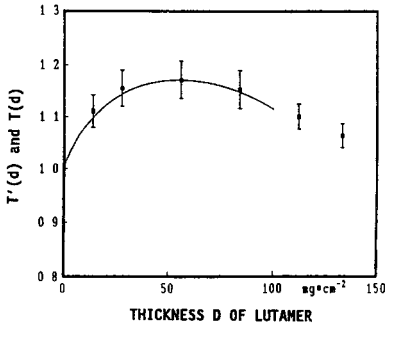


Figure 3. Dependence of the TSEE response T'(d) on the thickness of Lutamer covers (rectangles) in comparison to the transmission factors T(d) of tissue (line) for $^{90}Sr/^{90}Y$.

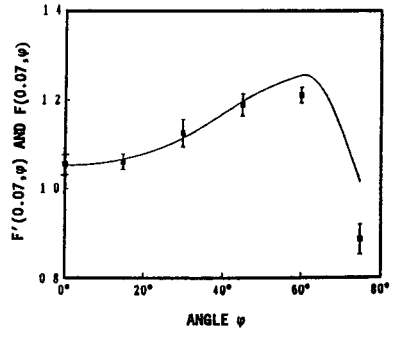


Figure 4. Angular response F'(0.07,φ) of detectors with Lutamer window to $^{90}Sr/^{90}Y$ (rectangles) in comparison to the conversion factors F(0.07,φ).

Head of Project 8: Dr M W Charles

II. Objectives for the reporting period

The overall aim of the project is to develop and validate methods for the measurement and calculation of doses from radioactive particulates ('hot particles'). During the current reporting period the objective was to extend measurements to include test sources of both Co-60 and a higher energy beta source Tm-170. A preliminary comparison was to be made between measurements and calculations. A revised improved design of Co-60 source was to be produced.

II.A. Objectives for the next reporting period

For the next reporting period, up to mid 1995, the main objectives will be to complete measurements of doses from Co-60 and Tm-170 test sources and to extend the measuring techniques to include thermoluminescence. The results of the various measuring laboratories will be combined and compared with calculations. Revisions will be made where necessary to the calculational methods. Recommendations will be given for the best methods of calculation and measurement of 'hot particle' doses. A Monte-Carlo code will be made available for selected sources and geometries. The limitations of the extrapolation chamber which have become apparent during this project will be reviewed and advice will be given on the corrections necessary if this method of measurement is preferred. The optimum methods will be applied to various practical sources such as 'hot particles' from Chernobyl. High dose rate Co-60 sources will be produced for pig skin studies of biological response.

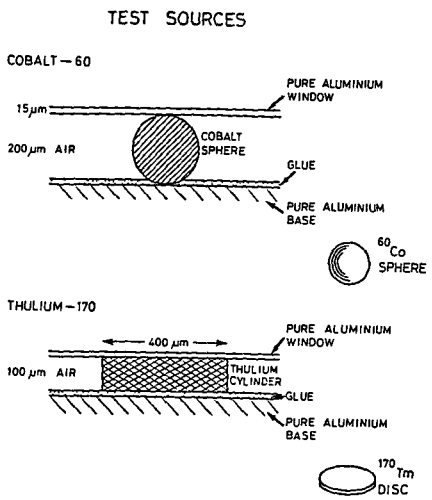
III. Progress achieved including publications

Previous work on this contract has been delayed due to difficulties of producing high specific activity neutron-activated sources in Europe. During the current reporting period Co-60 and Tm-170 sources have been produced at Petten and more recently at Brookhaven National Laboratory, USA. To date sources which have been produced are 200 μm diameter spheres of cobalt-60 with approximate activities of 8×10^5 Bq (20 μCi) and 8×10^6 Bq (200 μCi) and Tm-170 cylinders, 400 μm diameter and 100 μm thick, with approximate activities of 10^6 Bq (25 μCi). The source designs are shown in figure 1.

Doses have been evaluated for a Tm-170 source using an extrapolation chamber. Doses for one of the lower activity Co-60 sources have been determined in some detail using an automated extrapolation chamber at Berkeley and using radiochromic dye film at Berkeley and the National Institute of Standards and Technology (NIST), Washington DC (figure 2). Recent measurements on the same Co-60 source have been made using an extrapolation chamber, and thermoluminescence techniques at CEN Grenoble and Univ. of Montpelier. The measurements for all laboratories and for other sources are currently being carried out and collated.

Two calculational techniques have been used in this study: VARSKIN, a semi-empirical, PC-based, user-friendly code which uses an integration of Berger point kernels; PC-based Monte Carlo codes specially developed by Dr J Patau at Univ of Tolouse. VARSKIN gives a choice of nuclides and source geometries (Figure 3). The Monte Carlo codes use a well defined geometry in accord with the design of the test sources (figure 4)

Figure 1



DOSIMETRY:–

AUTOMATED SCANNING EXTRAPOLATION CHAMBER. TL (LiF, $CaSO_4$, Al_2O_3) CONVENTIONAL & LASER HEATING. RADIOCHROMIC DYE.

Figure 2

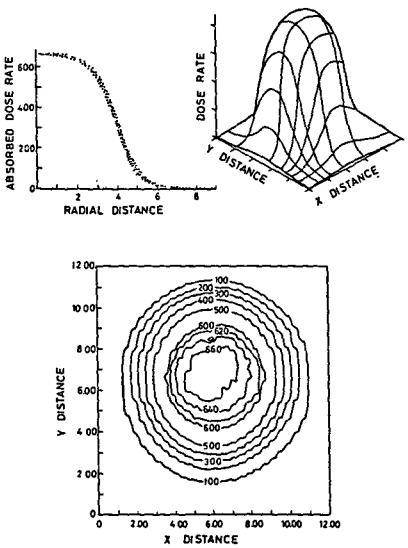
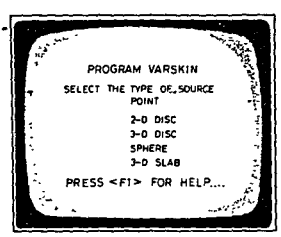


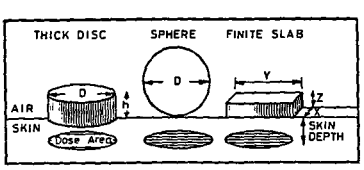
Figure 3

VARSKIN – MOD 2
(JAMES DURHAM, BATTELLE LABS)

BASED ON INTEGRATED BERGER 'POINT-KERNELS.' A USER FRIENDLY SOFTWARE PACKAGE DEVELOPED UNDER CONTRACT FOR NRC.



GEOMETRY OPTIONS SCREEN



THREE-DIMENSIONAL SOURCE MODELS

Figure 4

MONTE-CARLO CODE
TYPICAL GEOMETRY

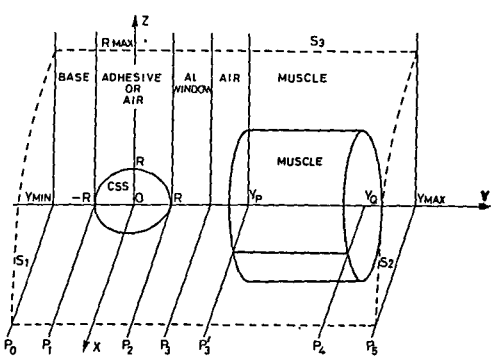
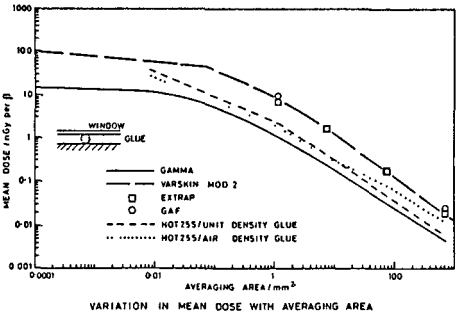


Figure 5 shows the results of dose measurements for cobalt-60 using an automated extrapolation chamber with various sized electrodes down to an area of 1.1 mm^2. The chamber window thickness was 12 μm mylar. The non-linear response of the chamber which has been described in earlier reports was corrected using a mathematical model to account for the non-uniform irradiation geometry. Measurements made using radiochromic dye film (GAF chromic) by using scanning laser (NIST) or image analysis (Berkeley) microdensitometry are also given. The agreement between all measuring methods is good. Measurements were a factor of 2-3 times greater than calculations made using an original version of VARSKIN (1992) but agree well with the calculations of the latest modified version, VARSKIN MOD2 (plus a gamma dose component calculated separately on the basis of a uniformly distributed spherical source model). The results of a preliminary version of a Monte Carlo code (HOT25S) specially developed by Dr Patau (Univ. Tolouse) are however a significant factor of up to 5 less than the measurements. The cause of this discrepancy is currently being evaluated by reviewing the calculational methodology and the source design. Monte Carlo calculations made using variations in the source design (Figure 5) are not greatly different so it is concluded that minor variations in source design should not be a major factor in this discrepancy. Nevertheless we have recently produced Co-60 sources of a slightly different design to ensure that the geometry is well characterised. Future work will aim to clarify this problem and will also include measurements using different techniques. The highest activity Co-60 sources will be used in a series of separately funded pig skin radiobiology studies.

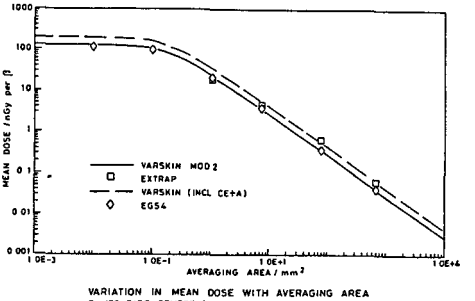
Figure 6 shows the results of dose measurements for Tm-170 using an extrapolation chamber (Berkeley) with a non-linear response correction. GAFchromic measurements are in progress. calculations are indicated for a Monte Carlo code developed by Dr Patau (which agrees with a version based on EGS4) and VARSKIN MOD2. Measurements and calculations agree well except for doses averaged over large areas. This discrepancy may arise from a small contributing dose from the stub on which the particle is mounted and this is currently being investigated. If conversion electrons and Auger electrons are included in the VARSKIN code then the dose is over-predicted. However we have identified an error in the VARSKIN code since it does not properly model absorption in the source for these low energy electrons (in fact they make very little extra contribution to the beta dose in this case). Future work with this source will include the use of other measurement techniques and modifications to VARSKIN (in collaboration with the originator J Durham at Battelle Labs, USA).

Figure 5



VARIATION IN MEAN DOSE WITH AVERAGING AREA
Co60 SPHERICAL SOURCE (200 μm DIAMETER)

Figure 6



VARIATION IN MEAN DOSE WITH AVERAGING AREA
Tm 170 DISC SOURCE (400 μm DIAMETER / 100 μm THICK)

Progress Report

Contract: **FI3P-CT920039** **Sector: A12**

Title: Development of instruments and methods for radiation protection dosimetry with the variance-covariance method.

1) A.M. Kellerer Univ. München
3) K.A. Jessen Univ. Aarhus-Hospital

I. Summary of Project Global Objectives and Achievements

This contract is aimed at the further development of twin detector instruments applicable in radiation protection practice. The participating groups are cooperating towards the development of improved methods to perform variance-covariance measurements, the development of twin-ionisation chambers, twin-proportional counters, and the electronics to operate the detectors.

In project 1 a multi-element proportional counter will be developed. The detector design follows ideas that have earlier been advanced by H.H.Rossi. Several prototype detector elements have been tested and their characteristics have been determined. The design best suited for ready and low cost construction of the multi-element detector will be chosen. The channels of the multi-element detector will be separated into two interdispersed sets, to function as a twin-detector. The detector development is accompanied by computational studies.

Project 3 deals with the development of improved detectors and improved methods of signal processing to perform variance-covariance measurements in high dose-rate beams. The efforts are focused on the measurement of dose averaged lineal energy in therapeutic and diagnostic x-ray beams and in therapeutic electron beams. Twin-ionisation chambers have been developed for this purpose. To cope with the high dose rate in these applications, signal processing and noise reduction had to be optimised.

Head of project 1: Prof. Dr. A.M.Kellerer.

II. Objectives for the reporting period

Proportional counters are, in principle, well suited to meet the requirements of increased precision and accuracy in radiation protection measurements. However their application in routine measurements necessitates better practicability of methods and instrumentation. In particular, there is lack of a small neutron detector with high sensitivity. Efforts have, therefore, been made in this project to develop a multi-element detector which is not too sophisticated in design for a production in substantial numbers. The work makes use of concepts that were first developed for multi-element detectors by H.H.Rossi.

III. Progress achieved, including publications

Two types of prototype detector elements have been produced and tested. The sensitive volume of each of the detectors is of cylindrical shape with diameter 3mm. The walls are made of A150 plastic material. A gold plated tungsten wire with diameter 20μm is used as central electrode in all cases. One type of these detectors has uninterrupted sensitive volumes. It has been build in a version that is 70 mm long, and , alternatively in a version that is 40 mm long. The second type of detector element has interrupted, separate sensitive regions. Its construction is diagrammatically represented in Fig.1. A special shape of the central electrode as shown in Fig. 1b was used; gas multiplication occurs only at the narrows intervals of the electrode, and 5 sensitive volumes of height 3mm are formed in this way. The counter utilizes insulating septa that separate the sensitive volumes (although they are perforated to allow for gas exchange) and support mechanically the central electrode (see Fig 1a.).

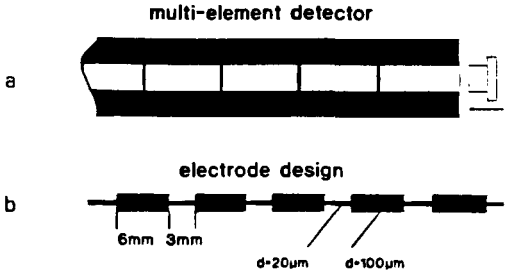


Fig.1: Schematic diagram of one detector 'channel' with 5 separate sensitive regions (a). The central electrode with segmented shape(b).

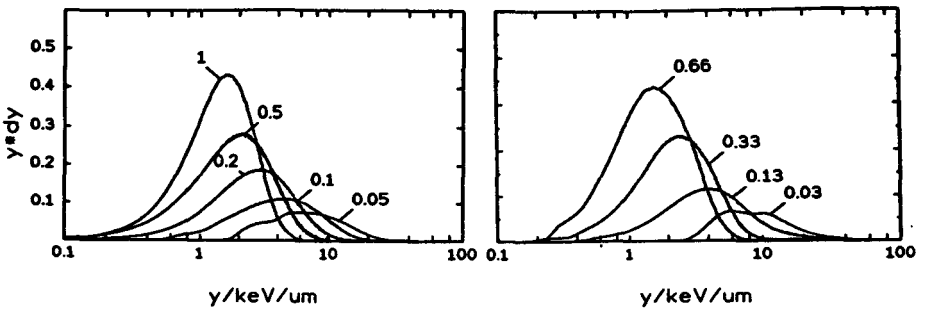


Fig.2: Single event spectra measured in Co-60 beams at various diameters of the simulated volume. The diameter is given by the figures at the graphs (μm). The height of the sensitive volume is 70mm (left panel) and 3mm (right panel).

The preparation of the electrode started with a gold plated tungsten wire of diameter 20μm. By galvanic coating the diameter was increased to about 100μm. With the use of a photolithographic process (coating with photoresist, UV exposure through a structured mask, development followed by etching), parts of the wire are reduced to their original diameter of 20 μm. The three prototype detectors were operated in a gas tight aluminium housing connected to a gas flow system. Methane based tissue equivalent gas was used.

In a series of measurements the characteristics of the detectors have been determined. Single event measurements, as well as variance-covariance measurements have been performed in Co-60 and in Cs-137 beams. Examples of the results are given in Fig.2.

In the next step of the development the design best suited for the purpose will be chosen. Numerous detector elements will be prepared to form a multi element detector.

Publications:

J. Chen, K.Hahn, H.Roos, and A.M.Kellerer, Microdosimetry of Therapeutic Electron Beams-Measurements and Monte-Carlo Simulations. Accepted for publication in Radiat. Prot. Dosim.

J.Chen, H.Roos and A.M.Kellerer, Microdosimetry of Diagnostic X Rays: Applications of the Variance-Covariance Method. Radiation Research 132, 271 (1992).

Head of Project 3: Dr. K.A. Jessen

II. Objectives for the reporting period.

Work has been undertaken towards:

- developing an improved twin-ionisation chamber designed to perform measurements of dose averaged lineal energy in diagnostic and therapeutic radiation beams.
- Further optimisation of integration time and noise reduction to improve the quality of the data.
- further adjustments and extension of computer programs for data processing.

Objectives for the next reporting period are the following:

- conclusions about the optimal mode of amplification.
- conclusions about the optimal noise reduction.
- further technical improvements towards a portable system applicable for quality assurance.
- finalising the computer programs.

III. Progress achieved, including publication.

Our work has been concentrated on measurements with a cylinder shaped detector pair in 260 kV conventional X-ray beams. The geometry, detector construction and experimental arrangement have been described earlier (1).

In order to optimise the signal size it has been decided, after careful considerations, to change the collecting electrode in the detector from one with a 0.15 mm diameter to one with 2.0 mm diameter. Voltage characteristics have been measured at the detector pressure: 7.5, 50.3, 100.0, 150.0, 200.0 mBar and a detector operating point on -400 V has been defined.

The change of electrodes did not lead to any significant improvement.

Measurements have also been performed with the WELLHOEFER IC5 ionisation chambers (range with WK92: 0.1 mGy/min - 199.99 Gy/min; sensitivity ($^{60}Co,D_w$): 2,7nC/Gy, sensitive volume 0.084 cm^3) under atmospheric pressure. These chambers are, however, not good for this type of experiment.

Test measurements have been done in relation to simulated diameters from 0.5 µm to 2.0 µm.
From the measured charged collected in the two detectors, a number of quantities are determined: the mean specific energy ($\bar{z}_1, \bar{z}_2\,[Gy]$), total relative variance ($\nu 1$, $\nu 2$), relative covariance ($C12$) and mean lineal energy (y_{D1}, y_{D2} [keV/µm]. '1' designates results determined with detector 1 and '2' designates results determined with detector 2.

The signal size has been checked via the measuring system.

By using dose tables from the 250 kV an approximate dose in the chambers has been calculated. This dose has been used to calculate the expected charge per collection and was found to be in accordance with the measurements. Also the dose has been compared with $\bar{z}$ for the simulated volume. $\bar{z}$ is calculated by the programme. In order to obtain the real dose, it is necessary to divide $\bar{z}$ by the ratio between the gas volume and simulated volume to the power of 2. When the real dose is corrected by this factor, the measured $\bar{z}$ value is found to be correct. It has, therefore, been concluded that the charge is measured correctly and the calculations are correct.

An adequate signal size has also been discussed.

The signal size is determined by adjusting the time period between each measurement. The longer the period, the more charge has been collected. The time period was adjusted by the internal clock frequency in the computer. This frequency had to bee 200 pulses per second at the very most, otherwise the DT2818 board would droop to such a great extent that the converted data would no longer be reliable. If the clock frequency is reduced, on will have to use an external clock.
Until this point, measurements have only been performed with the internal clock. However, changes have been made in the computer programme, and it is, therefore, possible from now on to collect charges in all time periods.

In order to obtain an assessment of a reasonable signal size, the results from variance-covariance measurements in pulsed therapeutic radiation beams have been used. By using the figures from (2), we have calculated each pulse to be in order of $1 \cdot 10^{-15}$C before amplification. Since our electrometers lowest measurement range goes up to 200 pC, it seems impossible to measure such small charges.

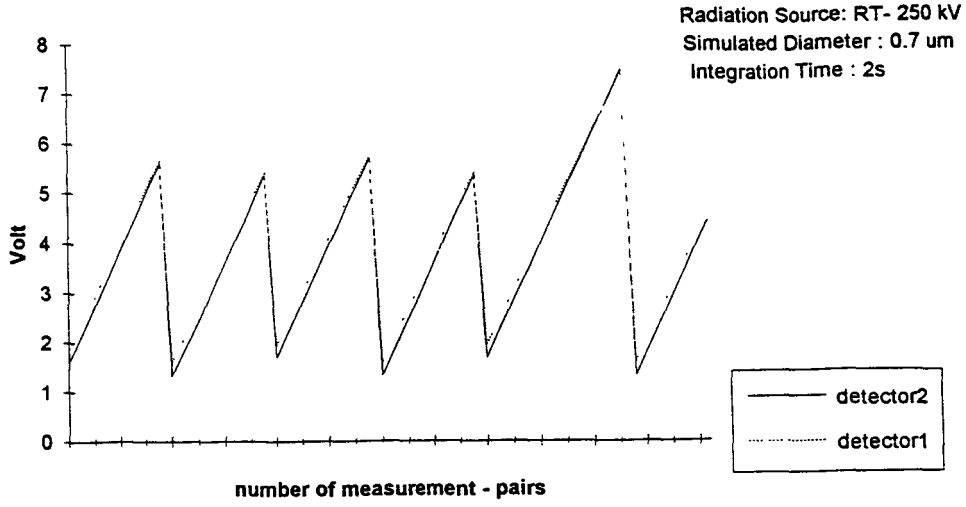


Fig.1. The signals from the detectors measured by electrometers as function of the number of measurement-pairs

Experiments with the collection time have been carried out.

In order to obtain a charge high enough to exceed the sensitivity of the electrometer, charge has to be collected for more than 2 seconds per pulse. Therefore the external clock was applied.

Since the collection time has to be so long, it is only possible to measure a few measurement pairs per pulse before the electrometers are overloaded. Because of this, the measurement procedure in our programme has been changed in order to measure repetitive pulse sequences (see fig.1.)

The measurements have been made with a collection time of 1, 2, 4, 6 seconds, and this renders such a large signal that the random variation begins to form a very even pattern. This means that even more points have to be determined in order to reach statistically reliable results, and the measurement time begins to be unrealistic. It follows that the signals from the chambers must be amplified before they enter the electrometers.

Further discussions with other contractors will be necessary, especially consultation with the Stockholm group, who have considerable experience with electrometers and amplifiers. Corporation on the same aspects of the work is also expected with new partners (PECO-program).

Publications:

1. Final Report Bi7-025, *Use of the Variance-Covariance Method in Radiation Protection.* Project 2, Dr. K.A. Jessen.

2. Honoré H.B., Jessen K.A., Nielsen H.H., *Variance-Covariance Measurements of the Dose Mean Lineal Energy in Beams for Radiotherapy,* Radiation Protection Dosimetry, Vol.31, No. 1/4 pp. 435-455 (1990).

3. Honoré H.B., Jensen, L.C., Jessen K.A., Nielsen H.H., *Variance-Covariance Measurements in Therapeutic and Diagnostic Radiation Beams of different Qualities,* submitted to Rad.Prot.Dosim., 1992.

Progress Report

Contract **FI3PCT920045**

Title: The use of microdosimetric methods for the determination of dose equivalent quantities and of basic data for dosimetry.

1)	Ségur	ADPA
2)	Brede	PTB
3)	Zoetelief	TNO-DELFT
4)	Schmitz	KFA Jülich GmbH
5)	Grillmaier	Univ. Saarlandes
6)	Barthe	IPSN-CEA-FAR

I. Summary of Project and Present Status:

The objective of this project is to develop and improve various detector systems for radiation protection dosimetry in neutron-photon fields. The techniques investigated are based on the measurement of absorbed dose and LET in order to determine the dose equivalent. Depending on the type of application, TEPC working at very low pressures and personal dosemeters will be developed. The dose equivalent response of TEPCs has to be determined in calibration fields with realistic spectra. Furthermore, to improve the calibration of individual dosemeters according to the new recommendations of ICRP, a transfer device for the determination of dose equivalent quantity must be developed

Finally, since the understanding and the usefulness of microdosimetric devices strongly depends on the basic knowledge of many varied microscopic physical quantities, special emphasis is devoted to the determination of basic data such as W values for neutrons and heavy charged particles, electron-molecule cross-sections in organic vapours, Kerma factors, etc..

a) Basic data for dosimetry :

a.1 Determination of W values for heavy charged particles (TNO):

In the case of heavy charged particles, simple analytical relationships have been used to fit measured data. W values for different particles (Proton, He, C, N, O) in methane based TE gas have been analysed in terms of this relationship.

a.2 Determination of electron-molecule cross-sections in organic vapours (ADPA):

The knowledge of electron-molecule cross-sections in organic vapours is of paramount importance not only for the theoretical study of the transport of electrons through TE gas but also

for the numerical modelling of the motion of electrons in the various counters investigated in this project. Cross-sections of isobutane and ethane were determined using a technique based on the unfolding of swarm parameters in gases. Furthermore a semi-analytical formula was developed to give the total ionisation cross-sections in various alkanes. It is now possible to predict, from the calculations, gas gain in isobutane or ethane based TE gas mixtures.

a.3 Determination of fluence-to-kerma conversion factors (PTB, Univ. Saarlandes):

Measurements were made at the Paul Scherrer Institut in Villigen (CH) in order to determine Oxygen and Nitrogen kerma factors. The spectral fluence of the fields for incident neutron energies between 2 MeV and 80 MeV was measured with a NE213 liquid scintillator by applying pulse-shape techniques for photon neutron separation and time-of-flight techniques for neutron energy determination.

a.4 Dose calculations in phantoms (PTB, KFA, TNO):

To establish a suitable combination of a TEPC and a phantom for use as a transfer device for dose equivalent, fluence-to-dose equivalent conversion coefficients are required in order to derive the reference values of dose equivalent from the primary physical quantity (particle fluence in the case of neutrons). Calculational optimizations of size and the materials of a phantom to be used in connection with a TEPC were performed (PTB).

Calculations of dose equivalents and of microdosimetric spectra in a male and female phantom have also been continued (KFA, TNO).

b) Improvement and developement of dosemeters based on microdosemetric principles:

b.1 Low pressure tissue equivalent proportional counter (Univ. Saarlandes, ADPA):

TEPC measurements in calibration fields with realistic spectra showed a satisfying dose equivalent response except in strongly moderated fields where the dose equivalent reading was too low. Decreasing the simulated diameter is the most suitable way to increase the dose equivalent response of TEPC to neutrons with energies around 100 keV without losing response for high neutron energies. The determination of the lowest pressure to be applied to the TEPC was made with the help of numerical calculation of the gas gain in the gas mixtures employed in the counter. During the design of a cylindrical TEPC, some practical problems were encountered, when decreasing the pressure, due to the occurrence of pulses in the high LET region (Univ. Saarlandes). To solve this problem numerical calculations of the electric field and determination of the motion of electrons inside this counter were undertaken (ADPA). In spite of this drawback, measurements performed with this newly developed detector in reference fields gave good agreement with respect to a spherical 1/2" detector.

b.2 Individual dosemeters (KFA, CEA):

b.2.1 Semi-conductor Detector (SCD):

Experimental investigation of various SCD was performed using SRAM (Static Random Access Memory), DRAM (Dynamic Random Access Memory) and GaAlAs (Gallium-Aluminium-Arsenate) detectors and the respective performances of these detectors were carefully compared to conventional results given by proportional counters (KFA).

b.2.2 Multi-cellular low pressure tissue equivalent proportional counter (MCPC):

Single channel and five-channels prototypes were designed and tested. The effect of the channel diameter on charge collection was investigated. The response of the counter to a monoenergetic neutron beam in the energy range 0.144 MeV to 2.5 MeV was determined for the first time.

Head of Project 1 : Dr. P. Ségur

II Objectives for the reporting period

Determination of electron-molecule cross-sections in isobutane and ethane.

Calculation of the electric field in 'real' proportional counters (Homburg counter and S/DOS multicellular counter).

Determination of the space and time variation of electron densities inside these counters and calculation of the associated gas gain for low pressures.

III Objectives of the next reporting period

Determination of the limits of operation of a proportional counter (pressure for which a counter is no longer proportional) depending on the geometry and on the gas.

Optimisation of the gas gain for these very extreme situations.

Determination of avalanche statistics and comparisons with experiment (Homburg).

Calculation of the LET spectra given by a counter at low pressures corresponding to incident monoenergetic electrons.

IV Progress achieved including publications

1-Determination of electron-molecule cross-sections in organic vapours

The knowledge of electron-molecule cross-sections is of paramount importance if we have to carry out numerical modelling of the motion of electrons in a proportional counter. These cross-sections are not only very important in determining the characteristics of the counter but also in calculating the energy deposited by high and low energy electrons in the gas during the slowing down process.

The few data being available for the moment in organic vapours and our objectives under this contract being to undertake calculations in tissue equivalent gases, we started some years ago a systematic determination of electron-molecule cross-sections in organic vapours. The first gas studied was methane. During the previous contract (BI7-030), we studied propane.

The techniques used to determine these cross-sections were detailed in the final report of contract BI7-030.

During the present contract, our work is mainly devoted to isobutane (i-C_4H_{10}) and ethane (C_2H_6) cross-sections.

One of the drawbacks, in the case of isobutane and ethane, is that, unlike most other gases, the total electron-molecule ionisation cross-section is not well known. Partial measurements exist for high and low energies, but they are not sufficient to give complete reliable cross-sections for all

organic vapours of interest to us. To solve this problem, we used the experimental results available, together with the following semi empirical relationship for the ionisation cross-sections $\sigma_{ion,n}$ of various organic vapours (in this work we will limit our investigation to alkanes):

$$\sigma_{ion,n}(\varepsilon) = A_n \frac{4\pi a_0^2 R}{\varepsilon} Log(1 + 0.08(\varepsilon - I_n))\phi_n(\varepsilon)$$

In the above relationship, ε is the kinetic energy of electrons and I_n is the ionisation threshold of the molecule. The $\phi_n(\varepsilon)$ function and constant A_n are specific to a given gas. A_n is determined using the high energy data available for ionisation cross-section and, furthermore, is directly connected to the oscillator strength. Table I shows the values of A_n obtained for the various alkanes.

Alkanes	A_n	M_i^2	I
CH_4	4.37	4.28	12.98
C_2H_6	9.02	8.63	11.65
C_3H_8	13.33	13.08	11.08
n-C_4H_{10}	17.57	17.80	10.5
i-C_4H_{10}	17.62	17.40	10.5
n-C_5H_{12}	24.61	24.40	10.33
i-C_5H_{12}	23.65	25.00	10.33
neo-C5H12	22.06	23.00	10.33
n-C_6H_{14}	34.23	34.80	10.17

Table I

Function $\phi_n(\varepsilon)$ must be determined from low energy experimental results for total ionisation cross-sections. Looking at the various experimental results, it appeared that only two different $\phi_n(\varepsilon)$ functions are necessary. The first one ($\phi_1(\varepsilon)$) must be introduced in the case of methane. The second one ($\phi_2(\varepsilon)$) is valid for all other alkanes. The following relationship shows the general analytical expression for the $\phi_n(\varepsilon)$ functions and the values of the corresponding constants are given in Table II.

$$\phi_n(\varepsilon) = a\,exp(-b/\varepsilon) + a'\,exp(-b'/\varepsilon^2) + a''\,exp(-b''(\varepsilon - I_n)/\varepsilon^2)$$

Figure 1 gave the results obtained from our formula compared with experimental data. These results show that it is now possible to obtain cross-section values in some energy ranges where there were no experimental results. Once the A_n constant is known for a given alkane, our formula allows the calculation of total ionisation cross-sections for the whole energy range (including relativistic effects). For example, it can then be seen that the use of this formula allowed the calculation, in isobutane, of low energy values of ionisation cross-sections which were missing in the literature.

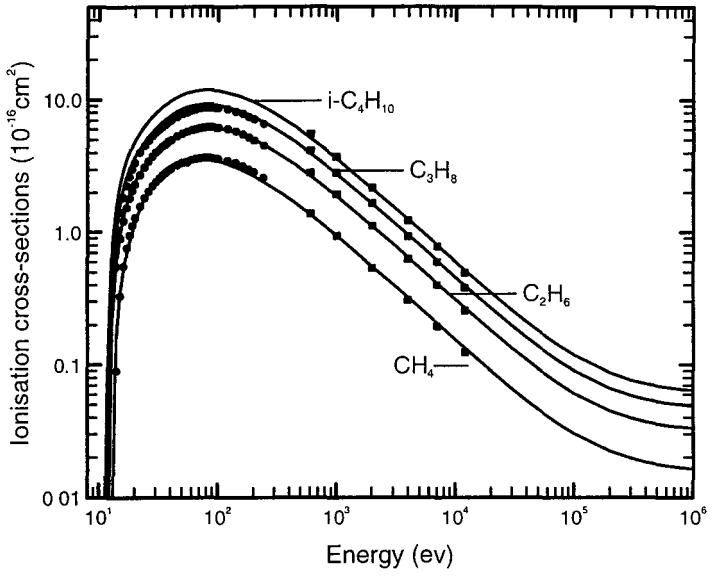


Fig.1a: Total ionisation cross-sections in alkanes
(Symbols) Experimental results (lines) Calculations

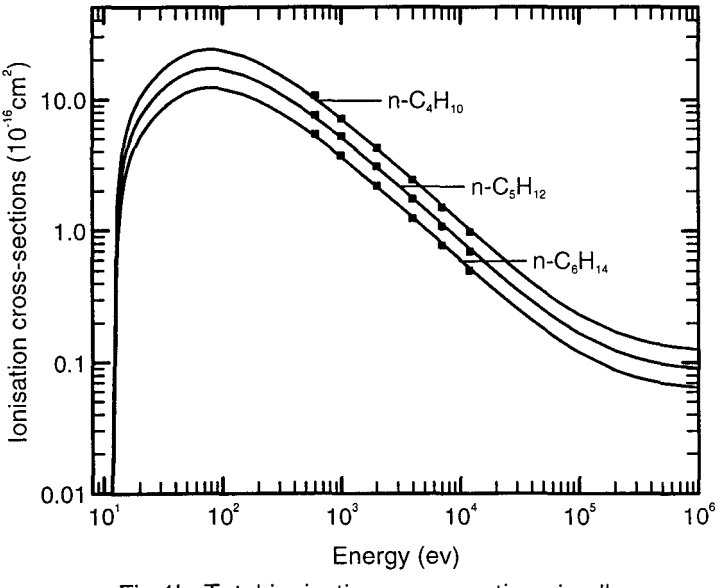


Fig.1b: Total ionisation cross-sections in alkanes
(Symbols) Experimental results (lines) Calculations

Besides ionisation cross-sections, we also determined most of the cross-sections corresponding to elastic and inelastic processes. Figures 2 and 3 show the two sets of cross-sections obtained in isobutane and ethane. Using these cross-sections, it is now possible to calculate the various swarm parameters (drift velocity, diffusion coefficients, ionisation coefficient, etc.) for pure gases or mixtures of gases which are of interest in the field of microdosimetry.

constants	$\phi_1(\varepsilon)$	$\phi_2(\varepsilon)$.
a	5.21	4.02
b	9.51	27.22
a'	-13.66	14.10
b'	23.48	-22.75
a"	9.50	-17.09
b"	-2.34	3.94

Table II

2-Numerical modelling of real counters

In most works, the numerical modelling of a proportional counter is limited to the calculation of the gas gain through the integration of the ionisation coefficient over the radial distance from the wire. The accuracy of this calculation can only be improved through a better knowledge of the ionisation coefficient. Although this procedure is usually well suited to give an order of magnitude of the gas gain in an 'ideal' proportional counter, it does not allow calculation of this gas gain in real situations since a counter is never perfect and strong field gradients may occur at the extremity of the anode wire (due to the existence of dielectric and metallic rings needed to fix the wire).

To calculate the gas gain in these conditions, it is necessary to determine first the real electric field inside the counter. The determination of the electric field is made numerically solving the Laplace equation. When there is a cylindrical symmetry (as in the Homburg counter), the space is assumed to be two dimensional. The main drawback in solving this problem is taking account the exact geometry of the counters including dielectric ring, guard rings, etc.

A numerical code was then developed which gives the two dimensional variation of the electric field for the Homburg counter and for some simplificated versions of S/DOS Multicellular Chamber.

Once the electric field is obtained, it is necessary to calculate the gas gain. The gas gain is now defined as the ratio between the total number of electrons collected at the anode to the initial number of electrons released in the gas. To determine the total number of electrons collected we solve, using the swarm parameters calculated with our electron-molecule cross-sections, the continuity equation. We then obtain the space and time variation of the number of electrons inside

the counter from the time when they were created inside the gas to the time when they completely disappear at the anode.

It is then possible to calculate the variation of the gas gain versus some parameters such as, voltage applied, size and shape of guard and dielectric rings, nature and pressure of the filling gas, position of the initial particles, etc. and, consequently, to optimise the geometry of the detector.

2.1 Homburg counter:

Figure 4 gives the 'optimum' electric field obtained for the Homburg counter.

2.2 Multicellular Drift Chamber:

Different simplified versions of this counter were considered. Figure 5 shows the spatial distribution of electric field in a cylindrical prototype. The counter geometry is very close to that of the Homburg counter except that now a Shonka plastic bored with channels is located inside the counter.

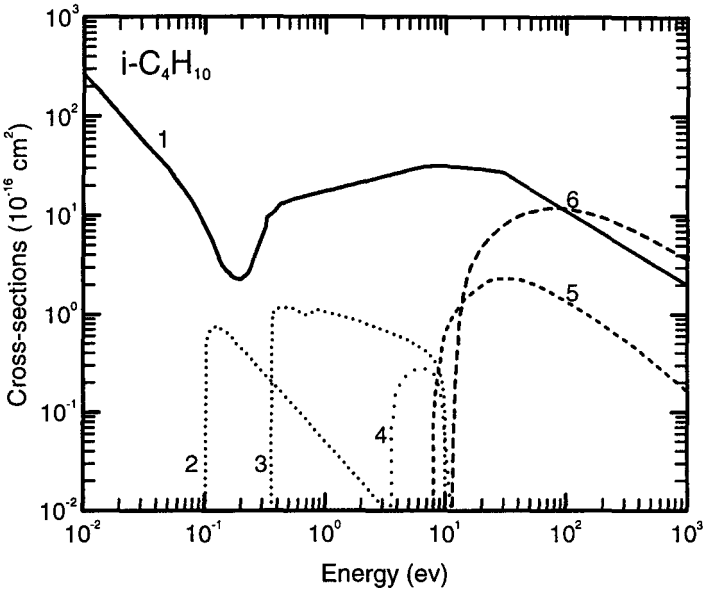


Fig.2 : Electron-molecule cross-sections in Isobutane
(1) Momentum transfer cross section (2,3) vibrations
(4) Dissociation (5) Excitation (6) Ionisation

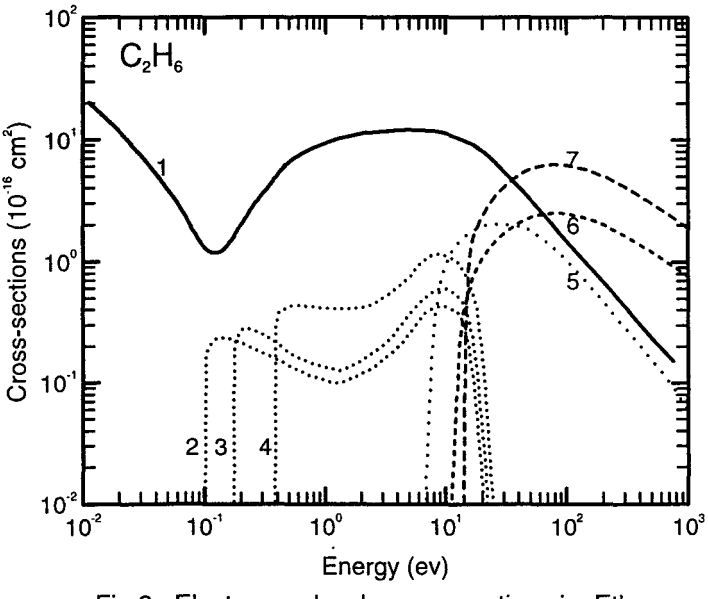


Fig.3 : Electron-molecule cross-sections in Ethane
(1) Momentum transfer cross section (2-4) vibrations
(5) Dissociation (6) Excitation (7) Ionisation

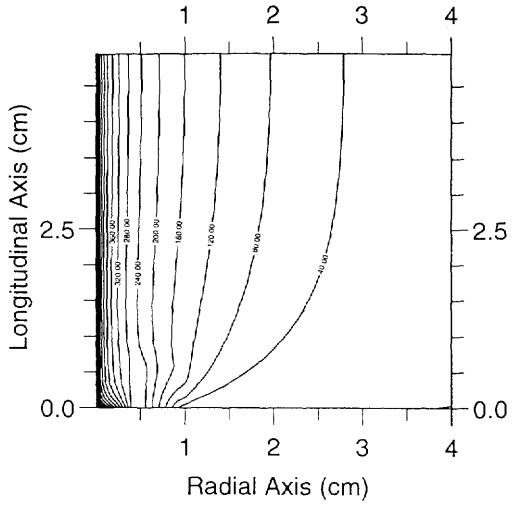


Figure 4
Equipotential lines
Voltage Applied 800 Volts, Radius of the Anode 0.005 micrometer
Radius of the cathode 2.53 cm

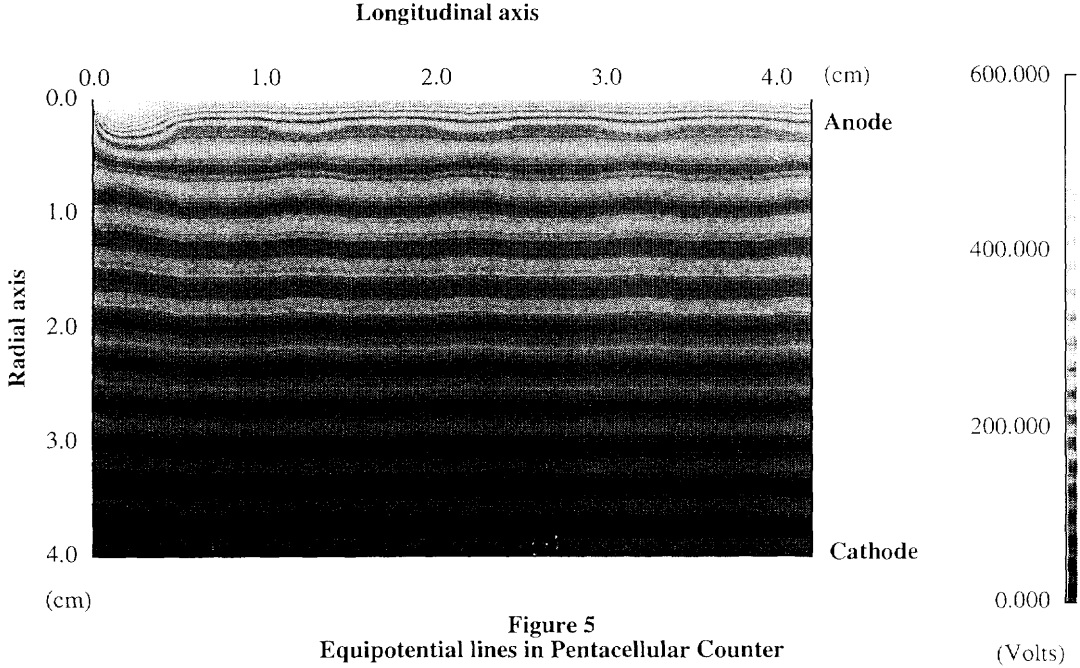


Figure 5
Equipotential lines in Pentacellular Counter

Publications

Gerdung S., Grillmaier R.G., Lim T., Pihet P., Schuhmacher H and Ségur P.: *Performance of TEPCs at low pressures: Some attempts to improve their dose equivalent response in the neutron energy range from 10 keV to 1 MeV,* Radiat. Prot. Dosim. in press (1993)

J. Barthe, M. Mourgues, J.M. Bordy, P. Segur, B. Boutruche et G. Portal.*Etude de compteurs miniatures pour la dosimétrie des neutrons.* Congrès IRPA Montréal 17-22 mai 1992.Vol. 1, pp. 479-482, Montréal.

D. Blanc, P. Segur, J. Barthe et J.M. Bordy.
Les compteurs proportionnels à milieu équivalent au tissu, en radioprotection. Congres IRPA Montréal 17-22 mai 1992. Vol. 1, pp. 459-462, Montréal.

B. Boutruche, J.M. Bordy, J. Barthe, P. Segur and G. Portal.
New concept of a high sensitive tissue equivalent proportional counter for individual neutron dosimetry. 11th Symposium on microdosimetry, Gatlinburg, Tennessee USA, September 1992

P. Ségur, A. Chouki, M. C. Bordage, J. Y. Gosselin, *Determination of electron-molecule cross sections in propane and calculation of swarm parameters in propane based mixtures,* XI Symp. Microdos. Gatlinburg, Ten., USA (Sept. 1992)

M. C. Bordage, J. Y. Gosselin, A. Chouki, P. Ségur, *Electron collision cross sections in Propane. Swarm parameters in propane and argon-propane mixtures*, ESCAMPIG, St Petersbourg, (Aout 1992).

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Legnaro Laboratories (Italia), Dr. Colautti
University of Prague (Czech Republic), H. Pruchova

Head of Project 2: Dr. Brede

II. Objectives for the reporting period

a) Improvements in neutron spectrometry with NE213 scintillation detectors
b) Development of a dose equivalent transfer device
c) Determination of ion yield spectra in oxygen and nitrogen

III. Objectives for the next reporting period

Calculational optimisation of the dose equivalent transfer device.
Determination of oxygen kerma factors above $E_n > 15$ MeV.
Determination of the neutron response of GM counters and Mg/Ar ionisation chambers for energies above 20 MeV.

IV. Progress achieved including publications

a) Improvements in neutron spectrometry with NE213 scintillation detectors

The consistency of spectral neutron fluence determination with hydrocarbon scintillation detectors using time-of-flight techniques and fluence measurements with a proton recoil telescope, has been investigated for neutron energies of 44 MeV and 65 MeV [1].
If the full response of the scintillation detector, including contributions from n-C reactions, is used for the fluence determination, the results differ up to 15 % from the measurement with a proton recoil telescope. The deviations depend on the detector threshold and the neutron energy.
Fig. 1 shows a comparison of an experimentally determined response function and a calculation with the Monte Carlo code SCINFUL at En = 50.5 MeV.
An explanation of this deviation may be due to an inadequate description of the n-C reaction channels and the cross section data sets available in the Monte Carlo SCINFUL code that has been used to calculate the response and the efficiency of the scintillator.

b) Development of a dose equivalent transfer device

The study of a suitable combination of a TEPC and a phantom for use as a transfer device for dose equivalent has been continued. As a basis for these investigations, fluence-to-dose equivalent coefficients are required in order to derive the reference values of dose equivalent from the primary physical quantity (particle fluence in the case of neutrons). With the new ICRP recommendation on the quality factor, conversion coefficients from fluence to ambient dose equivalent, H*(10) [2,3], to directional dose equivalent, H'(10) [4] and to personal dose equivalent, Hp(10) [4] were recalculated.

Further calculational optimizations of size and material of a phantom to be used in connection with a TEPC were performed. If a phantom material is used with macroscopic scattering and absorption cross sections similar to ICRU tissue, dose equivalent is overestimated by about 30 % for neutrons with energies below 50 eV. The reason is the higher nitrogen content of A-150 plastic (used as wall material for the TEPC) compared to ICRU tissue. This overestimation can be compensated - without significantly modifying the properties at higher neutron energies - by selecting a phantom which leads to a smaller fluence of thermal neutrons in the position of the TEPC, e.g. by chosing a phantom with a different hydrogen content, density or size. Calculations of a TEPC in a slab have been started in order to study the angular dependence of such a device compared to that of Hp(10).

c) Determination of ion yield spectra in oxygen and nitrogen

First experiments have been performed at the Paul Scherrer Institut (PSI), Villigen, CH, in order to determine oxygen and nitrogen kerma factors. The spectral fluence of the neutron field has been measured with an NE213 liquid scintillation detector [1] by applying pulse-shape techniques for photon neutron separation and time-of-flight techniques for neutron energy determination. Special cavity chambers with walls made of Al, Al_2O_3 and ALN developed at the University of Birmingham, U.K. and of Zr and ZrO_2 developed at the University of Wisconsin, Madison, U.S.A. have been used. The difference techniques have been applied in order to extract the spectra for the pure elements aluminium, zirconium, nitrogen and oxygen.

Fig. 2 shows the results of an analysis at En = 45 MeV. The difference in the spectra corresponds to the contribution of oxygen for both detector systems. Otherwise the oxygen ion yield spectra from Al/Al_2O_3 measured with PTE gas differ from those from Zr/ZrO_2 measured with $Ar+CO_2$ gas. From a comparison with the ion yield spectra of carbon measured with the respective gases, it can be concluded that this difference is almost exclusively due to the different gas-to-wall dose conversion coefficient.

Fig. 3 shows the comparison of the nitrogen and carbon ion yield spectra at E_n = 45 MeV. All measurements were performed with PTE gas. To a certain extent the ion yield spectra of nitrogen and carbon agree in the region where alpha particles are predominant but they show a great difference in the proton region. This implies that the $^{14}N(n,p)^{14}C$ reaction cross section is significantly greater than the $^{12}C(n,p)^{12}B$ reaction cross section in this neutron energy region.

Publications

[1] R. Nolte. H.J. Brede, U.J. Schrewe, H. Schuhmacher.
Neutron Spectrometry with Liquid Scintillation Detectors at Neutron Energies between 20 MeV and 70 MeV: A Status Report, Physikalisch-Technische Bundesanstalt: PTB-N-9 (June 93) ISSN 0936-0492

[2] H. Schuhmacher and B.R.L. Siebert. *Quality Factors and Ambient Dose Equivalent for Neutrons Based on the New ICRP Recommendation.* Radiat. Prot. Dosim. 40, 85-89 (1992).

[3] H. Schuhmacher, R.A. Hollnagel and B.R.L. Siebert. *Sensitivity Study of Parameters Influencing Calculations of Fluence-to-Dose Equivalent Conversion Coefficients for Neutrons.* Submitted to Radiat. Prot. Dosim.

[4] B.R.L. Siebert and H. Schuhmacher. *Calculated Fluence-toDirectional and Personal Dose Equivalent Conversion Coefficients for Neutrons*. Submitted to Radiat. Prot. Dosim.

Scientific staff:

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Other research groups collaborating actively in this project:

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Univ.Catholique de Louvain-la-Neuve, Unit.Phys.Nucl.(Prof. J.P. Meulders) (B) University of Goettingen, 2. Phys. Institut (Prof. F. Smend) (D)

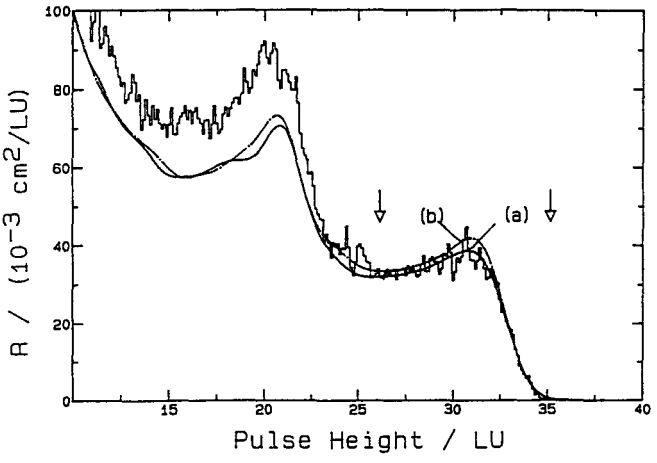

Fig.1: Experimental response spectrum (histogram) for En = (50.5 q 0.5) MeV, (LU: light output unit). Curves (a) and (b) are calculations with the SCINFUL code (details see [1]). The arrows indicate the pulse-height region used for the normalization of the calculated spectra to the experimental response.

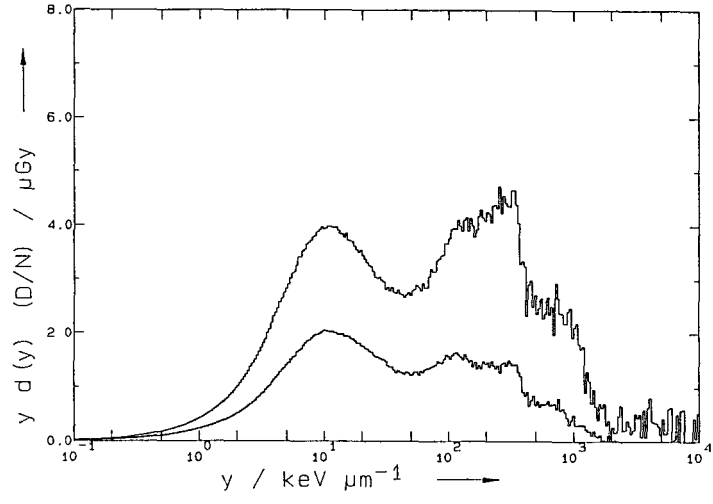

Fig. 2a: Ion yield spectra in Al 2O3 and Al. Both spectra were normalized to the counts N of the neutron fluence monitor. The Al2O3 spectrum was multiplied by the factor (1/wAl) = 1.889 so that the difference between the spectra corresponds to the contribution of oxygen.

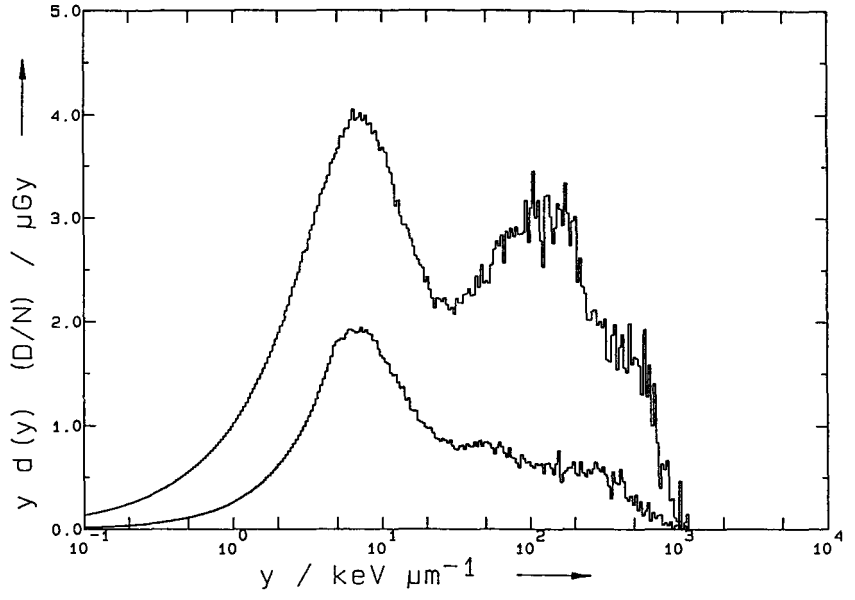

Fig. 2b: Ion yield spectra of ZrO 2 and Zr. Both spectra were normalized to the counts N of the neutron fluence monitor. The ZrO2 spectrum was multiplied by (1/wZr) = 1.351 so that the difference between the spectra corresponds to the contribution of the ion yield spectrum in oxygen.

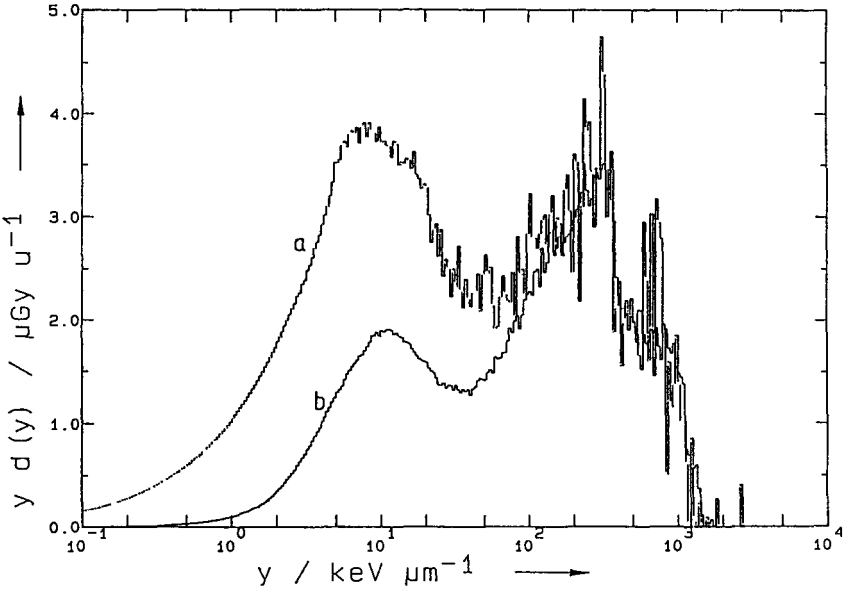

Fig. 3: Ion yield spectrum in nitrogen (a) from the difference of AlN and Al detectors. The ion yield spectrum in carbon (b) is shown for comparison. All measurements were performed with PTE gas.

Head of Project 3: Dr.H. Zoetelief

II Objectives for the reporting period

The irradiation facilities at the University of Louvain-la-Neuve are essential to explore basic data and detector characteristics at neutron energies in excess of 20 MeV. TNO will contribute to the characterization of these neutron fields as planned for by PTB through measurements with ionization chamber and GM counters.
TNO will contribute to the activities of Eurados Working Group 10 collaborating on improvement of basic data for neutron dosimetry and detector construction. Emphasis will be placed on improvement of W-values for neutron dosimetry.
Calculations of neutron transport with the MCNP code will be started with the aim of using this code together with the Caswell/Coyne (NIST) analytical code for studying the response of detectors inside various radiation fields in terms of operational dose equivalent quantities.

III. Objectives for the next reporting period.

Measurements at the high energy neutron beam at the facilities of UCL will be performed to determine the sensitivities of Geiger-Müller (GM) and Mg/Ar ionization chambers to neutrons with energies above 20 MeV. The experiments will be carried out in close collaboration with UCL and PTB simultaneous with our measurements. PTB will use time-of-flight techniques for the specification of the mixed neutron/photon fields. TNO will continue to contribute to the activities of EURADOS Working group 10. Calculation of neutron transport with the MCNP code on organ doses in broad beams of mono-energetic neutrons will be continued. The use of MCNP together with the Caswell/Coyne (NIST) code for studying the response of detectors in various radiation fields in terms of operational dose equivalent quantities will be started.

IV. Progress achieved including publications

The measurements for characterization for the high-energy neutron beams at the University of Louvain-la-Neuve (UCL) are not yet performed. Most likely experiments will be made in November 1993 in close collaboration with PTB and UCL.
Up to now, the use of the MCNP code has been restricted to calculations of organ and effective doses in case of external photon and electron beams employing anthropomorphic phantoms developed by GSF for adult male (ADAM) and female (EVA) (Kramer et al., GSF Bericht S-885, 1982). To obtain confidence in the use of the MCNP code for calculation of neutron transport, effective doses are being calculated employing ADAM in broad beams of monoenergetic neutrons. The results are compared to those of Hollnagel (Rad. Prot. Dosim., 44, 155-158, 1992).
Emphasis has been placed in the present contract period on the improvement of W_n-values, i.e. the mean energy expended per ion pair formed, for neutrons. For proportional counters or ionization chambers used in neutron dosimetry, the ion yield or specific ionization may be converted into

energy loss or absorbed dose in the gas by means of an average W-value. This average concerns a variety of charged particles generated in wall and gas.

Up to now, various general reports on W-values are available or in preparation including ICRU-31 (1979), Goodman and Coyne (1980), Rubach and Bichsel (1982), Burger and Makarewicz (1984), Burger et al. (1985), Coyne et al. (1990) and IAEA (in preparation). In ICRU-31 emphasis is placed on W-values of charged particles and various gases. Goodman and Coyne (1980) calculated W_n for neutron energies between 0.1 and 20 MeV for A-150 plastic ionization chambers with infinite cavities for methane based tissue equivalent (TE) gas using evaluated published information on W-values for charged particles. Rubach and Bichsel (1982) performed calculations of W_n in the neutron energy region from 0.4 to 14 MeV for different cavity sizes and A150/methane based TE-gas and C/CO_2 chambers. The activities at GSF (Burger and Makarewicz, 1984; Burger et al., 1985) concerned an evaluation of W for charged particles and calculation of W_n for neutrons with energies from thermal to 20 MeV in A-150/methane based TE-gas and C/CO_2 ionization chambers with various cavity sizes. Coyne et al. (1990) employed information from Huber et al. (1985) and Waibel and Willems (1985) on W-values of charged particles for calculation of microdosimetric spectra for low energy neutrons. The IAEA coordinated research programme on atomic and molecular data for radiotherapy focusses on providing more recent information on W-values for charged particles including validity of additivity rules for mixtures of gases.

The present work is aimed at providing an update of existing information on W_n. As a first step, an analysis is made of W-values for charged particles based on more recent information, e.g. Huber et al., 1985.

Concerning W-values for protons in methane-based TE-gas, recently a new analytical representation was presented by Siebert et al. (Microdosimetry Symposium, Gaithersburg, USA, 1992). They proposed the following analytical expression for the W-value of protons as a function of energy ($W_f(E_p)$):

$$W_f(E_p) = A\{\ln(E_p + B)\}^{-D} + CE_pE_b/(E_p^2 + E_b^2) + W_\infty \qquad (1)$$

where E_p is the proton energy, W_∞ the W-value at infinite proton energy, the parameters A, B, and D are similar to those used in an older expression suggested by Coyne et al. (Rad. Prot. dosim. 31, 217-221, 1990) and the constant C and cofactor are used to model a _bump" at a proton energy near E_b. The subscript *f* was introduced to have a value of 1 for a model without a bump, i.e., C=0 and a value of 2 for a model with a bump. The parameters and uncertainties (one standard deviation) are given in table 1 for the model with bump.

For the analysis of W-values for a, C, N and O particles, equation (1) has been rewritten:

$$W_R(E_R) = A\ \{\ln(E_R + B)\}^{-D} + C\ E_R\ E_b\ /\ (E_R^2 + E_b^2) + W_\infty \quad (2)$$

where E_R is the specific energy in keV per nucleon for the particle (R) under consideration.

particle	*A (eV)*	*B*	*C (eV)*	*D*	E_b*(keV)*	W_∞*(eV)*
p	16.56 ± 0.39	1.043 ± 0.034	2.01 ± 0.19	1.653 ± 0.054	300	29.10 ± 0.11
He	36.2 ± 0.3	1	4.1 ± 0.6	0.817 ± 0.010	201±2	23
C	44.1 ± 0.6	1	0	0.799 ± 0.015	-	23
N	43.3 ± 0.4	1	0	0.715 ± 0.006	-	23
O	46.3 ± 0.5	1	0	0.737 ± 0.012	-	23

Table 1. Analytical fits for W-values of charged particles as a function of specific energy in methane based TE-gas according to equation (2).

Experimental data on W-values of α-particles in methane based TE-gas were used from Huber et al (Rad Res. 101, 237-251, 1985), Nguyen et al. (Phys. Med. Biol. 25, 509-518, 1980), Whillock and Edwards (Phys Med. Biol. 28, 367-374, 1983), Thomas and Burke (Phys. Med. Biol., 30, 1215-1223, 1985), Rohrig and Colvett (Rad. Res., 76, 225-240, 1978), Varma and Baum (Phys. Med. Biol. 23, 1162-1172, 1978), Kemmochi (Health. Phys. 30, 439-446, 1976) and Krieger et al. (Phys. Med. Biol. 24, 286-298, 1979). The uncertainties stated by the authors vary from 0.3 per cent (e.g., Rohrig and Colvett) to approximately 3 per cent (e.g. Nguyen et al.). The error discussion by the various authors differs greatly in detail. Some authors only include statistical uncertainties whereas others give an estimate and error propagation of statistical as well as systematic errors. Some of the results were obtained relative to other gases whereas other investigators performed absolute measurements. The smaller uncertainties quoted seem not to be realistic. Fits to all data according to equation (2) employing a relative uncertainty of 1.6 per cent results in a p-value of 0.48. The resulting value of B of 1.14 is not significantly different from 1. The value of the parameter B minus one might be physically interpreted as the specific energy at which the production of ion pairs is no longer possible. Since this interpretation seems not realistic B was fixed to one. With this fixed value of B the p-value of the fit reduces only slightly to 0.40. A fit without a "bump" i.e. C=0 results in a p-value of 6.10^{-3}. This suggests that similar to the situation for protons a bump around a specific energy of about 200 keV/nucleon is realistic.

Experimental data on W-values of C-, N- and O-particles in methane based TE-gas are less abundant than for protons or α-particles. Data for W-values of carbon were obtained from Huber et al (1985), Rohrig and Colvett (1978) and Chemtob et al (Phys. Med. Biol. 23, 1197-1199, 1978). W-values for nitrogen were taken from Huber et al (1985) and Chemtob et al (1978). For W-values for oxygen, data were used from Huber et al (1985), Nguyen et al (1980) and Varma et al (Radiat Res, 70, 511-518, 1977). The uncertainties quoted by the authors vary from about 1 to 3 per cent. Fits to the data allowing for a "bump" result in negative W_∞-values for nitrogen and oxygen and a

C-value not significantly different from zero for carbon. Therefore fits were made for the model without bump (i.e., C=0) and a fixed value for B equal to one and a relative uncertainty of 3 per cent for the data. Resulting p-values for C, N and O were 0.40, 0.995 and 0.69 respectively. This might lead to the conclusion that errors for N are estimated too large. However, W_∞-values were quite different i.e. 27.7±1.8 eV for C, 15.5±1.5 eV for N and 22.7±1.8 eV for O. The largest specific energies investigated are quite different, i.e. for oxygen by far the largest value was included in the measurements, i.e. 2569 keV/nucleon, whereas the maximum values for C-and N-particles are only 53 and 27 keV/nucleon respectively. Since the W_∞-values for α particles and O-particles were not significantly different, it was decided to fix W_∞ for all particles to 23 eV.

particle	*relative standard deviation (%)*	*p-value*
p	2.5	-
a	1.6	0.40
C	3	0.21
N	3	0.18
O	3	0.75

Table 2. Uncertainties in the fits presented in Table 1

The results of fits of W-values for protons (Siebert et al), α-, C- N- and O-particles (present work) are summarized in Table 1 for methane-based TE-gas as a function of specific energy. An indication of the uncertainties is given in Table 2.
To improve the existing information experimental studies might be conducted at larger specific energies for C- and N-particles. In addition measurements could be performed to provide some explanation for the bump in the proton and a-particle data.

Scientific staff

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Other research groups collaborating actively in this project:

Eurados Working Group 10 (mainly Prof. J.P. Meulder and Dr. G. Taylor)

Head of Project 4: Dr. Th. Schmitz

II Objectives for the reporting period

- Experimental investigations of semiconductor detector properties in neutron fields, using SRAM (Static Random Access Memory), DRAM (Dynamic Random Access Memory) and GaAlAs (Gallium-Aluminium-Asenate) chips as microdosimetric detectors
- Optimisation of the detector design with respect to the thickness and the geometry of the sensitive voxels for charged secondaries from neutron radiation
- Investigation on the relation of microdosimetric spectra measured with semiconductor detectors and tissue equivalent counters
- Calculation of dose equivalents in anthropoid male and female phantoms for incident neutron and gamma radiation fields
- Calculation of microdosimetric spectra in selected organs in anthropoid phantoms
- Determination of radiological risk based on the concept of biological response functions

III Objectives of the next reporting period

- Further experimental investigations on the suitability of DRAM and GaAlAs chips as microdosimetric detectors in mixed neutron gamma radiation fields
- Simulation of chord length distributions in semiconductor detectors
- Further investigations on the consequences of the recommendations of ICRP in its report 60
- Evaluation of biological response functions, relevant for radiation protection

IV Progress achieved including publications

Experimental investigations of semiconductor detector properties in mixed neutron gamma fields have been performed, using SRAM (Static Random Access Memory), DRAM (Dynamic Random Access Memory) and GaAlAs (Gallium-Aluminium-Arsenate) detectors. The experiments with the SRAM chips were performed in a collimated fast neutron beam, which is produced by the bombardment of a Beryllium target with 14 MeV deuterons. Microdosimetric spectra evaluated from the results of these measurements show characteristic features, which can be interpreted on the basis of the experimentally determined thickness and location in the chip of the sensitive elements, calculated secondary charged particle spectra and on the basis of the comparison to proportional counter experiments.

The comparison of dose values measured with the SRAM chips and with proportional counters in the same field differ by about a factor of ten. The calibration of the SRAM chip is performed with an 241Am source either in terms of energy or in terms of lineal energy. No special correction is as yet introduced to correct the response of the detector with respect to absorbed dose. The major disadvantage of the SRAM chip is that no information on the actual layout of the chip is known, which results in uncertainties in the value of the mean chord length used in the determination of

lineal energy. Furtheron, at least two types of sensitive elements have been identified in the chip, which are of different geometrical shape an are located at different depth in the chip.

First experiments have been performed with DRAM (Dynamic Random Access Memory) chips inneutron fields. The results of these measurement show that such elements may be well suited as microdosimetric detectors for mixed neutron-gamma radiation fields. Therefore, a cooperation has been set up with the Fraunhofer Gesellschaft, Institut flr mikroelektronische Schaltungen (FHG IMS). This institute has gathered a lot of experience in the detection and spectroscopy of alpha radiation with modified DRAM chips. The final design of a chip suited for microdosimetric measurements has been worked out and it is expected that a first prototype will be available in fall 1993.

Finally Gallium Aluminium Arsenate (GaAlAs) diodes, which show the effect of internal amplification, have been tested with the Americium alpha source and in the neutron beam at the cyclotron of the KFA. This detector concept offers the chance to simulated sub micron volumes or to decrease the lower detection level in order to increase the sensitivity to photons. Another advantage of the Gallium Arsenate technology is the use of high doping levels, which imply steep and high potential walls, i.e. geometrically well defined sensitive elements.

The calculation of dose equivalents and of microdosimetric spectra in a male and female phantom have been continued. The Monte Carlo transport code MNCP has been used for the necessary transport calculations. Two phantoms have been developed for this code, a male phantom on the basis of the ADAM phantom and a female phantom on the basis of the EVA phantom. Both phantoms (ADAM and EVA) were modified in the sense that they contain all organs, for which weighting factors are given in the last ICRP recommendations, i.e.: gonads, red bone marrow, colon, lung, stomach, bladder, liver, oesophagus, thyroid, skin, bone surface, and remainder. The tissues and their material composition follow exactly the MIRD-5 specifications, except for some trace elements which have been neglected.

Dose equivalents, using the quality factor definition of ICRP 60, and equivalent doses have been calculated for both phantoms for 33 incident neutron energies and different geometries, e.g. AP, PA etc.. The results of these calculations have been transferred to the PTB with the aim to include them into the data set, which will used by a joint ICRP-ICRU committee to determine amongst other things fluence to dose equivalent conversion functions.

The results of the MCNP calculations were in addition used to calculate microdosimetric distributions in different organs of the phantoms. The size of the assumed sensitive sites were 1 5μ, 8 5μ and 20 nm. In order to evaluate the radiological risk in such organs, the microdosimetric spectra were folded with biological response functions for some selected endpoints. E.g., for red bone marrow (RBM), the response function for abnormal metaphases determined on the basis of biological data from Skarsgard et. al. (1967) was applied. The resulting risk estimates per unit incident fluence can be compared to conventional measures of the radiological hazard, such as equivalent doses in particular organs, HT. The dependence of the ICRP defined equivalent doses on neutron energy cannot be justified on the basis of these phenomenologically derived risk estimates.

The investigations show that microdosimetric distributions are useful for characterising radiation quality variations within complex biological systems and that they can be applied for predicting radiological risks, i.e. the probabilities of specific low level radiation effects in particular organs. There is however an obvious need for response functions derived from radioepidemiology and from in vivo animal experiments, such as leukaemia induction.

Publications

Pierschel, M., Ehwald, K.-E., Heinemann, B., Januschewski, F., Schmıtz, Th., Schrvder, O. *A BCCD-based Dosemeter for mixed Radiation Fields..* Sixth European Symposium on Semiconductor Detectors, 24th - 26th February 1992, Milano, Italy, IEEE Trans. Nucl. Instr. Meth. (in press).

Morstin, K., Kopec, M., Schmitz, Th. *Equivalent Doses vs. Dose Equivalents for Neutrons Based on New ICRP Recommendations.* Radiat. Prot. Dosim., 44, 159-164 (1992).

Morstin, K., Kopec, M., Schmitz, Th. *Microdosimetry in Anthropoid Phantoms* Radiat. Prot. Dosim (in press).

Booz, J., Morstin, K., Kopec, M., Olko, P., Schmitz, Th., Feinendegen, L.E. *Bidimensional Microdosimetry as a Tool for Evaluating Biological Response and Target Structure.* In: Biophysical Modelling of Radiation Effects (Chadwick, K.H., Moschini, G., Varma, M.N. edts.), Adam Hilger New York, 163-170 (1992)

Schrvder, O., Schmitz, Th., Pierschel, M. *Microdosimetric Dosemeters for Individual Monitoring Based on Semiconductor Detectors* Radiat. Prot. Dosim. (in press).

Schrvder, O., Schmitz, Th. *Can a personal dosemeter for neutron radiation based on a semiconductor chip match The new ICRPrecommendations* Individual Monitoring of Ionizing Radiation: The Impact of recent ICRP and ICRU Publications, Vıllingen, May 5 - 7, 1993

Scientific staff

D. Barthmann, L.E. Feinendegen, H.-W. Mlller-Gdrtner, Th. Schmitz, O. Schrvder
M. Kopec, K. Morstin (AGH Krakau), P. Olko (INP, Krakau)

Other research groups collaborating actively in this project:

- Members of EURADOS working Committee 10
- Fraunhofer-Institut flr mikroelektronısche Schaltungen und Systeme, Institutsteil Dresden

Head of Project 5: Dr. Grillmaier

II. Objectives for the reporting period

To construct a TEPC for use in radiation protection and to investigate the operational characteristics for use of TEPCs with very low pressures.
To determine the dose equivalent response of TEPCs in neutron-gamma fields with energy spectra similar to those found in realistic environments.
To measure the kermafactors in Oxigen for neutrons of 50 and 70 MeV (in close collaboration with PTB).

III. Objectives for the next reporting period

To construct a series of cylindrical TEPCs for use in radiation protection based on the prototypes built in the institute. To test the long term stability and to investigate the calibration of detectors with cylindrical geometry.
To continue the calculation of gas gain in collaboration with Toulouse.
To continue the measurements of the dose equivalent response of TEPCs in realistic fields. It is forseen to combine these measurements with new methods developed at other institutes collaborating in the frame of the contract.
To determine the kermafactors in Oxigen for neutron energies of 40 and 60 MeV.

IV. Progress achieved including publications

A large volume cylindrical TEPC has been constructed in the laboratory. The aim of the project is to build an operational instrument for radiation protection purposes. Beside robustness which is necessary for routine work, a large volume is required to ensure a sufficiently high sensitivity for low dose environments. Due to the large volume the gas pressure simulating a diameter of 2 µm commonly used is rather low (900 Pa). This makes high demands on the vacuum tightness and the electrical design because of the low electrical insulation of the employed gases at these pressures. The electrical design could be optimised by experimental studies and with the help of computer simulations of the electrical field inside the detector in collaboration with the university of Toulouse. One remaining problem concernig the construction of TEPCs is the raise of pulses in the high LET region the reason of which is not yet fully understood. Although the number of such pulses per unit time could be suppressed sufficiently to be negligible in fields with high dose rate, they still represent a problem for routine use of the detector in many practical environments because of their generally low dose rate.
The dose equivalent response of TEPCs is close to one for environments where photons and high energy neutrons are predominant. However, in strongly moderated neutron fields (energies aorund 100 keV) its response is too low due to the short range particles. Decreasing the simulated diameter

offers the most suitable way to ameliorate the dose equivalent response of TEPCs to neutrons with energies around 100 keV without changing the good response for the high neutron energies[1]. The investigation of the operational characteristics of TEPCs at pressures simulating less than one micrometer is partly connected to the above described problem of high LET pulses. Decreasing the pressure without changing the electrical field strength results in an augmentation of the number of such high pulses. Moreover these pulses are clearly related to radiation. Investigations to clarify this phenomenon have been started in collaboration with the university of Toulouse by simulating the gas gain processes and thus defining the theoretical limits for the lowest possible pressure to be applied to Proportional counters with a given geometry. At the same time efforts are undertaken to improve the construction of the detector with special care to the cleaning and assembling procedures. The work will be continued in the frame of this contract.

Measurements with the new developed detector have been performed in reference fields at the PTB, Braunschweig. The detector was exposed to a ^{60}Co photon field and to a ^{252}Cf neutron field. The shapes of the measured microdosimetric distributions are in good agreement with such of conventional spherical TEPCs. Slight differences in the low and the high LET part are assumed to be a consequence of differences in the chord length distributions in a sphere and in a cylinder. With regard to the energy dose the new developed detector overestimates the expected value by about 20 %. As this factor remains constant in the different radiation fields the difference might be due to the size of the sensitive volume which is assumed to be defined by the field shaping tubes. This might not meet the real conditions and therefore result in a wrong mass of gas. To confirm this assumption further investigations are needed.

The facility at CEN/CEA in Cadarache/France simulates neutron spectra which are typically encountered in 'realistic' environments such as around nuclear industries. Measurements with TEPCs have been performed in order to determine their dose equivalent response to mixed n-g fields with broad neutron energy distributions. The measurements have partly been done with the new developed detector. Moreover the spectral neutron fluence has been measured and calculated by other groups allowing to extend the interpretation of the measured microdosimetric spectra by means of Monte-Carlo simulations.

In the field of Cadarache the dose equivalent reading of the TEPC ranges from 80 % to 60 % depending on the degree of moderation of the primary neutrons. Figure 1 shows microdosimetric distributions measured in the same configuration of the facility with the new developed cylindrical and two spherical TEPCs filled at 2 and 0.5 µm simulated diameter.

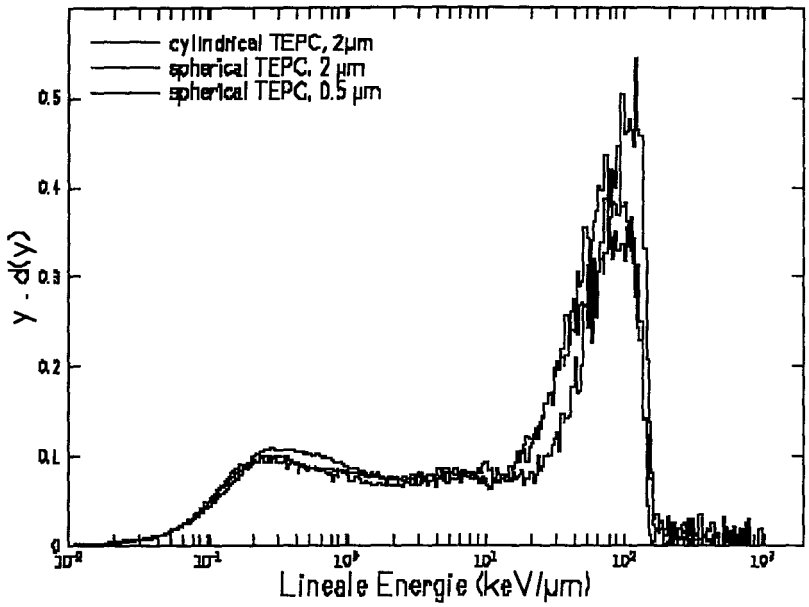


Figure 1: Microdosimetric distributions measured at the Cadarache facility with the new cylindrical detector and a 1/2" spherical detector filled to 2 and 0.5 µm simulated diameter.

The preliminary measurements with the 1/2" detector simulating 0.5 µm diameter of tissue leaded to the expected amelioration of the dose equivalent reading by a factor of about 10 %. The effect of the lower simulated diameter leads to a larger dose fraction in the region of low energy protons (about 100 keV/µm) and to a small 'peak' at 10 keV/µm. The measurements with the cylindrical TEPC confirmed the results obtained at the PTB. The difference observed between the spherical and cylindrical detector might be due to the above mentioned differences in the chord length distributions but it could also result from the wrong mass of gas corresponding to a different simulated diameter.

The measurement of kermafactors has been continued in close collaboration with the PTB (see earlier reports). For the energies of 50 and 70 MeV the kermafactors in Oxigen and Nitrogen have been measured using the difference of energy absorption in a pure metal and its compounds with Oxigen or Nitrogen.

Publications

Gerdung S., Grillmaier R.G., Lim T., Pihet P., Schuhmacher H and Ségur P.: *Performance of TEPCs at low pressures: Some attempts to improve their dose equivalent response in the neutron energy range from 10 keV to 1 MeV,* Radiat. Prot. Dosim. in press (1993)

Scientific staff

M. Freyermuth, S. Gerdung, Pr. Grillmaier, T. Lim

Other research groups collaborating actively in this project:

Members of EURADOS Working Group 10
Batelle Pacific Northwest Laboratories, Richland (Dr. J. Mcdonald, USA).
Commissariat à l'énergie Atomique, CEA Fontenay aux Roses (Dr. J. L. Chartier, F. Posny)
Centre d'Etudes Nucléaires, CEN cadarache (Dr. Mourgues)
Paul Scherrer Institut, PSI Villigen (Dr. R. Henneck, Dr. P. Schmelzbach)

Head of project 6 : J. Barthe

II Objectives for the reporting period.

The main objectives, envisaged for the reporting period, are :

- Design, realisation and experimentation of single and five-channel prototypes associated with a wire anode in order to demonstrate how the Multi-Channel Tissue Equivalent Proportional Counter (MC-TEPC) would have to work.

- Design and realisation of the first multi-channel counter and its response in reference neutron beams. Calculation codes on the electric field configuration are developed in collaboration with the CPAT research team headed by P. Ségur and reported in his contract.

III Objectives for the next period.

The first MC-TEPC built in our laboratory is very simple (without any guard ring), so it was not possible to achieve consistent data on low lineal energies. The objectives, planned for the next period of the contract, concern mainly :

- the improvement of the MC-TEPC geometry and of its associated electronics,

- the measurement of the angular and energy responses on phantom in reference neutron beams according to the ICRP 60 recommendations.

IV Progress achieved including publications.

1 - Description of the single and five-channel prototypes.

In order to validate the multi-cellular geometry, a single and a five-channel prototype have been designed and tested. The prototype (figure 1) employs a stack of epoxy resin plates, covered with a thin tin-copper layer and provided with either a central hole or a row of five holes. A biased resistance bridge is used, to generate a constant electric field into the drift region inside the channel. The counter is filled with methane at a 100 torr pressure. A ^{238}Pu alpha source induces ionisation in the gas volume between the cathode and the first electrode. The secondary electrons are subjected to the electric field and drifted through the channel towards the multiplication region. A 25 µm diameter anode wire made of tungsten and a channel depth of 1 cm were used for both prototypes.

2 - Experimental set-up.

The experimental set-up used consists of the two counter prototypes, a Canberra 2006 preamplifier, two Siléna 7611 linear amplifiers and a two-way Inel Cicero 4K multi-channel analyser (2*2048). Counters are emptied by the means of an ultra-vacuum pump until 10^{-5} Torr and filled with high-grade gases.

3 - Experimental results.

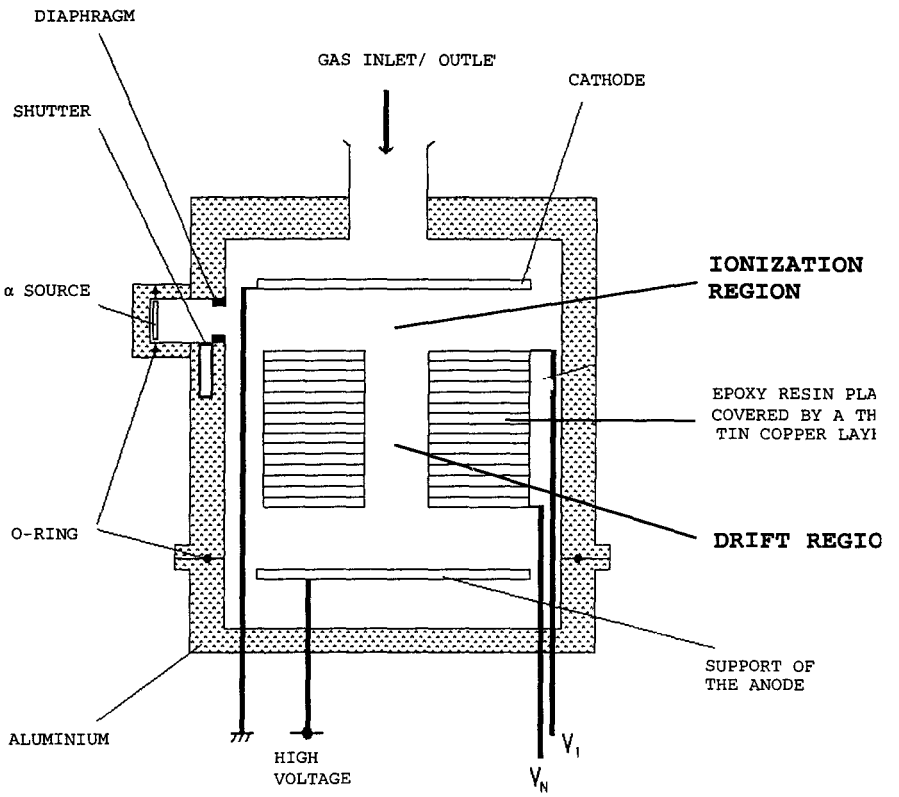


Figure 1 : Cut view of the single-channel prototype.

Two sets of experiments were performed to study the effect of the channel diameter (0.5 and 1 cm) and of the channel number (1 and 5) on the collected charge.

For each type of experiment, drift and multiplication regions were investigated. When the drift region was investigated, the potential in the multiplication region was maintained constant and the difference in potential applied between the channel extremities varied. The opposite procedure was used to investigate the multiplication region.

Figures 2 shows the collected charge evolution in the drift and multiplication regions for the two different channel diameters. the collected charge observed for the drift region (a [1 cm] and b [0.5 cm] curves) increases rapidly with the anode voltage below 450 V (a 100 volt difference in potential being applied to the drift region). This can be explained by the enhanced focusing effect on electrons coming from the ionisation region towards the channel. Above 700 V (a 350 volt difference in potential being applied to the drift region), the collected charge observed decreased

slightly for the 0.5 cm diameter channel. This can be explained by a greater number of electrons collected by the first electrode. In the multiplication region (c [1 cm] and d [0.5 cm] curves) an increase of the collected charge with the applied potential was observed. The collected charge was higher for the smallest diameter; this could be due to a greater electric field in the drift region.

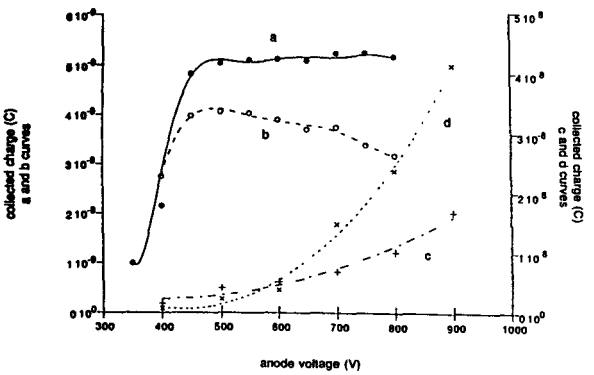


Figure 2 : Collected charge in the drift (a and b) and multiplication (c and d) regions for the two counter diameters.

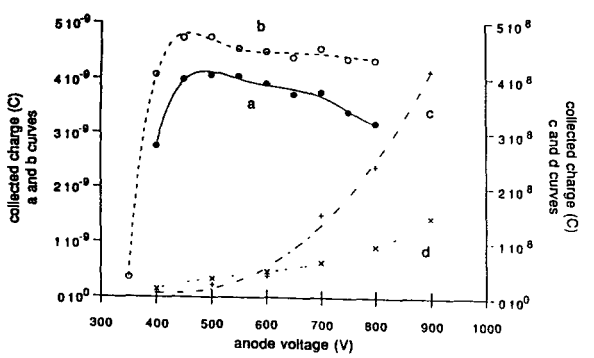


Figure 3 : Collected charge in the drift (a and b) and multiplication (c and d) regions for 1 and 5 channel counters.

Figure 3 shows for the two different prototypes, the collected charge variation in the drift and multiplication regions. In the drift region (curves a and b), the observations are close to those shown in figure 2 (curves a and b). The collected charge is greater for the larger number of channels but the respective ratio is not proportional to their number. This paradoxical behaviour

could be explained by the assumption of overlapping effects in the ionisation region. An increase of the collected charge with applied voltage is observed in the multiplication region (curves c and d); nevertheless, the lowest collected charge corresponds to the largest number of channels. This seems to be due to the alpha beam collimation angle in the experimental set-up.

4 - Description of the MC TEPC.

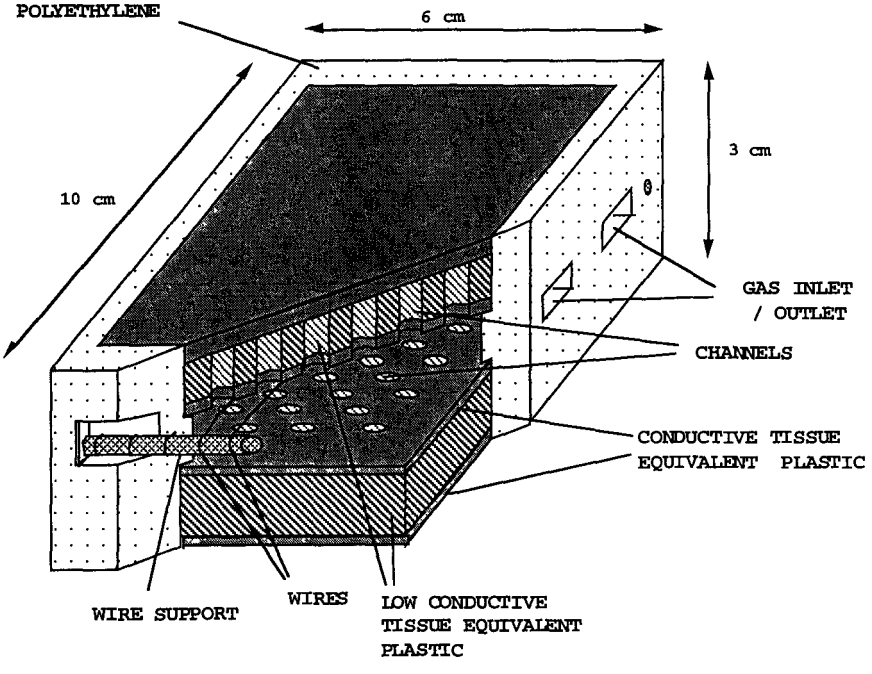


Figure 4 : 3D view of the MC TEPC

The multicellular counter prototype (figure 4) is made of two different tissue equivalent plastics : an A150 conductive plastic used to generate the isopotential surfaces and a tissue equivalent plastic with a low electric conductivity used to generate an electric field inside the channels. The two cathodes together have about 250 cylindrical holes. The anode wires are centred in front of each hole row at the same distance from the two cathodes. A low positive voltage is applied to the punched faces and a high voltage to the anode. A polyethylene plate keeps the cathodes and wire supports in position.

5 - Mains theoretical characteristics of the MC TEPC

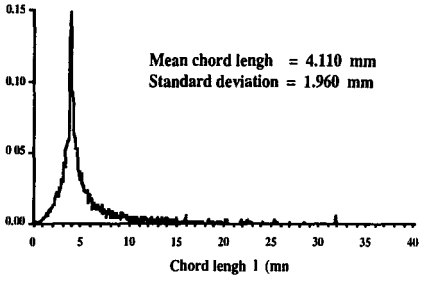


Figure 5 : Chord length distribution of the multi-channel counter.

The use of a common TEPC, as sensor in a personal dosemeter, meets great difficulties mainly due to its low sensitivity. This leds us to investigate a new design of counter based on the multicellular geometry intended to increase the radiating surface of the cathode. This is obtained by using two flat cathodes in which many holes are drilled. A 0.2 cm distance was chosen both for the radius of the holes and their separation. The splitting surface increase is significant with respect to the spherical and ortho-cylindrical volumes (table 1).

Table 1

Internal diameter (sphere or ortho-cylinder)(cm)	0.5	1.0	1.5	2.0	2.5	3.0
Surface area ratio w.r.t. a sphere	848	212	94	53	34	24
Surface area ratio w.r.t. an ortho-cylinder	565	141	63	35	23	16

A Monte-Carlo code was written to evaluate the chord length distribution of the counter and checked for spherical and cylindrical counters; a good agreement with the theoretical values was obtained (difference less than 0.1%). Figure 5 shows the chord length distribution obtained. The mean and the most probable chord length are identical (within 1%). This is an important advantage for microdosimetric calculations because, in this case, the lineal energy spectrum is close to that of LET even for a low radiation count number statistic.

6 - Experimental results.

The MC-TEPC was tested free in air with mono-energetic neutron beams within the energy range of 0.144 MeV up to 2.5 MeV. Figure 6 shows a few representative spectra. Due to the high background signal of the counter (lack of guard rings), these spectra do not present a lineal energy response below 10 keV/m.

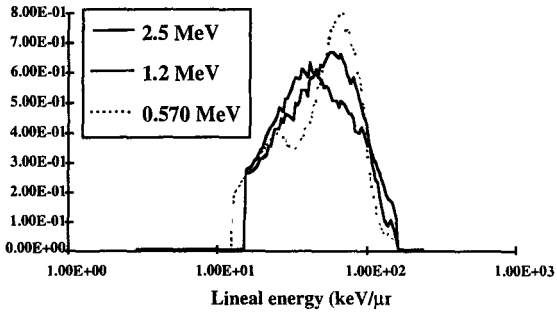


Figure 6 : Energetic fluence spectra for different neutron energies.

Publications :

J. Barthe, M. Mourgues, J.M. Bordy, P. Segur, B. Boutruche et G. Portal. *Etude de compteurs miniatures pour la dosimétrie des neutrons.* Congrès IRPA Montréal 17-22 mai 1992. Vol. 1, pp. 479-482, Montréal.

D. Blanc, P. Segur, J. Barthe et J.M. Bordy. *Les compteurs proportionnels à milieu équivalent au tissu en radioprotection.* Congres IRPA Montréal 17-22 mai 1992. Vol. 1, pp. 459-462, Montréal.

B. Boutruche, J.M. Bordy, J. Barthe, P. Segur and G. Portal. *New concept of a high sensitive tissue equivalent proportional counter for individual neutron dosimetry..* 11th Symposium on microdosimetry, Gatlinburg, Tennessee USA, September 1992

J.M. Bordy. *Contribution à la réalisation d'un compteur proportionnel à dérive équivalent au tissu destiné à la dosimétrie individuelle en radioprotection.* Rapport CEA -R-5603, Service de documentation, CE Saclay 91191 Gif sur Yvette Cedex France, juillet 1992.

Scientific staff

B. Boutruche, J. M. Bordy, J. Barthe

Other research groups collaborating actively in this project:

T. Waker, CNRL, Chalk River (Canada)

Progress Report

Contract : **FI3P-CT930072** **Sector : A12**

Title : Individual electronic neutron dosemeter.

1)	Vareille	Univ. Limoges
2)	Zamani-Valassiadou	Univ. Thessaloniki
3)	Barthe	CEA - FAR
4)	Fernandez Moreno	Univ. Barcelona - Autonoma
5)	Curzio	Univ. Pisa

I - Summary of Project Global Objectives and Achievements

The contract deals with individual neutron dosimetry in accordance with new recommandations. Two types of dosemeter are concerned :

- *Electronic device*

Thanks to electronic possibilities this real time-dosemeter will be able to calculate and give the "exposure story" of any people, then compare it to the official recommandations. Various improved devices have to be proposed studied and tested.

- *Superheated drop detector*

The aim is to obtain an immediate reading.

In order to realize an accurate dosemeter it is necessary to identify the best combination of superheated material (or mixture of materials) and thermodynamic conditions.

The two types of dosemeter will be characterized through : the response to direct neutron beams, the neutron energy fields in which the response is sufficient to give valuable informations, the limits of detection, the on phantom response, uncertainties on the measured dose equivalent and rate.

Two parts are proposed :

- a separate study of theoritical conceiving and realization of the two devices in order to improve them.

- an experimental comparizon of the two devices thanks to joint irradiations.

Head of project 1 : Prof. VAREILLE and Prof. DECOSSAS

II - Objectives for the reporting period

The main objectives for the reporting period are :

- design and realization of new devices (square PIPS detector, no metallic parts...).
- electronic tests and measurements on the detector.
- beginning of realization of computer codes for the new devices.

III - Objectives for the next period

Thanks to the device which is now realized electronic measurements in various neutron and γ fields in LEPOFI (sources) well be done in order to improve the device. Calculations taking into account the phantom contribution will be obtained and the results compared with the experimental ones. Joint irradiations mainly with monoenergetic felds, with other contractors, will probably take place in March 1994 in order to characterize the devices.

The first proposal concerning the model of a multi-area system will be presented (calculations and technological considerations [LAAS - Toulouse]).

IV - Progress achieved including publications

*Taking into account the results already obtained (during the contract B107-020) [1,2] a new device has been designed.

In order to reduce the γ-contribution [3] the metallic part of the device is very reduced ; the diode is placed in plastic box. Metallic parts consist of thin evaporated Al layer of 0.1 μm thickness.

The geometry of the detector is also changed, i.e. the PN junction is a square one the area of which is 225 mm^2.

The bias voltage is now of 8 V, with a depleted zone of 30 μm thickness. The junction capacity lies between 640 pF and 860 pF.

New devices are now realized and have to be tested. Unfortunately, because it was not possible to order new diodes before official acceptation of the contract by the Commission, the working time was too short and we are not able to give here results of electronic tests and measurements which are now in progress.

* Thanks to the simulation code writen at LEPOFI, a model of calculations is elaborated for the new device. Using Monte-Carlo method and EGS4 code, the calculations are in progress ; some statistical problems have been solved. Albedo contribution is to be taken into account through experimental results from litterature which are now collected.

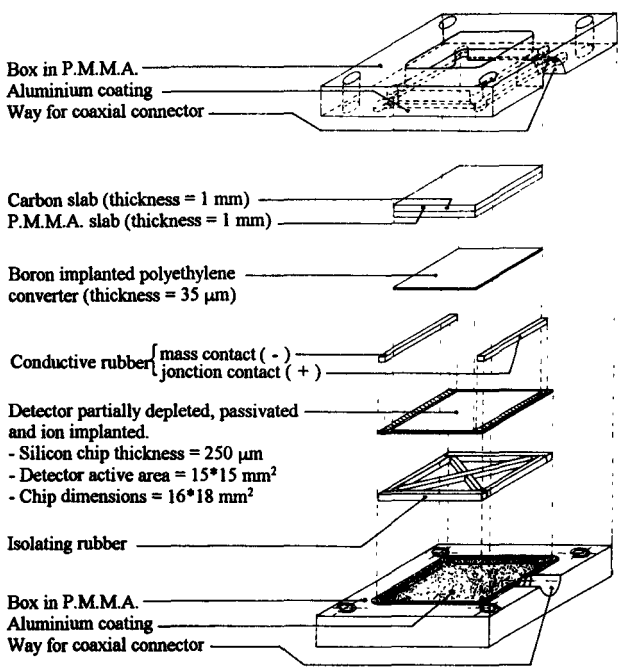
* An investigator of UAB (Barcelona) has spent 3 months (April, May, June) in LEPOFI (Limoges) to work on the existing electronic system in order to study electronic methods.

References

1 - BARELAUD B., PAUL D., DUBARRY B., MAKOVICKA L., DECOSSAS J.L, VAREILLE J.C.
Principles of an electronic neutron dosemeter using a PIPS detector - Radiat. Prot. Dosim., 44, 363-366, (1992).

2 - BARELAUD B., DUBARRY B., PAUL D., MAKOVICKA L., DECOSSAS J.L, VAREILLE J.C.
Evolution du capteur électronique pour la dosimétrie des neutrons développés au LEPOFI -
Radioprotection - to be published.-

3 - PAUL D.
Perturbation gamma dans un dosimètre neutronique à diodes
Thesis - 14-1992 - Université de Limoges (1992).



Head of project 2 : Dr. ZAMANI-VALASSIADOU

II - Objectives for the reporting period

The new converter consisting of ^{6}LiF evaporated on CR 39 was applied on Si diodes. The response of the system was studied at thermal neutrons as well as at 2.5 MeV and in Am-Be neutron field. Irradiations with gamma rays were made in order to compare with gamma contribution coming from neutron reactions on the diode mount.

III - Objectives for the next period

The new converter will be applied to Limoges diodes as well as to other electronic dosimetric systems. The energy response and the linearity of the systems is also planned to be examined.

IV - Progress achieved including publications

Up to now the experience of our group on neutron dosimetry is mainly coming from SSNT Detectors. During the last years we had the opportunity to develop a new converter based on the $^{6}Li(\alpha, n)$ reaction and proton recoils. The dosimetric properties of the new system (converter-detector) were studied during Eurados-Cendos Joint irradiations and also in the frame of B17 0020C CEC contract (Bruyeres le Chatel, Cadarache). Tests in various neutron energies and doses were performed and the characterizations of the system were found to be very interesting for Radiation Protection purposes.

Now the same converter can be adapted to various electronic dosimetric systems which are able to count light particles such as alphas, protons etc.

At first the performance of a Silicon surface barrier detector was studied with the new converter. The Si detector in close contact with LiF radiator evaporated on 500 μm CR 39 (converter) was fixed in the preamplifier. Output is connected with linear amplifier and output pulses are fed into a multichannel pulse height analyser in order to get the pulse height spectrum. The detector was operated by applying a voltage of 20 V.

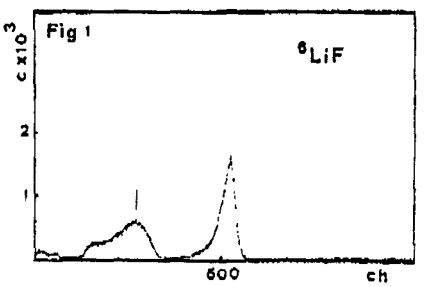
Measurements were performed at a first step in the Nuclear Reactor.

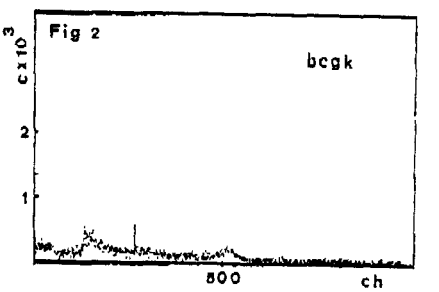
A calibration with ^{241}Am source was made in order to compare with alpha particle line coming from the reactions on the LiF radiator with neutrons. In figure 1 the spectrum is seen with alpha peak in channel 270 and with triton peak in channel 530. In figure 2 we give the same spectrum without LiF radiator, which is the background of the counting system.

A constant threshold at channel 130 was applied in order to reduce gamma contribution from reactions mainly coming in the mount of the detector. This value was derived from experimental data . For this reason the Si detector spectrum was studied after irradiation with ^{137}Cs and ^{22}Na gamma sources.

By intergrating over the total spectrum a neutron response of the order of 100 counts/μSv was obtained.

Irradiations with Am-Be source were also performed. The spectrum is presented in figure 3. The two peaks of alpha particles and tritons are larger than in the termal neutron cases. Events at higher enregies are also observed as it was expected from reaction kinematics. However, the neutron response in this case is estimated to be of the order of 0.5 counts /μSv. The same order is found by irradiating at 2.5 MeV neutrons from the neutron generator.





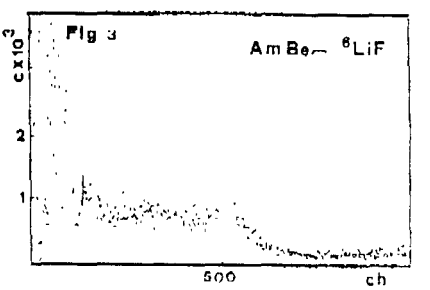


Figure Captions.

Fig. 1. Spectrum taken by Si diode with ^{6}LiF radiator on CR39(converter) at the Nuclear reactor.

Fig. 2. The same spectrum without converter, taken as background for the experiment.

Fig. 3. Spectrum taken by Si diode with the converter by irradiating in Am-Be source.

Head of project 3 : Dr. BARTHE

II - Objectives for the reporting period

The main objectives, envisaged for the reporting period, are :

- design and realisation of three double diodes,
- background and electronic tests,
- response under photon and neutron source radiation.

III - Objectives for the next period

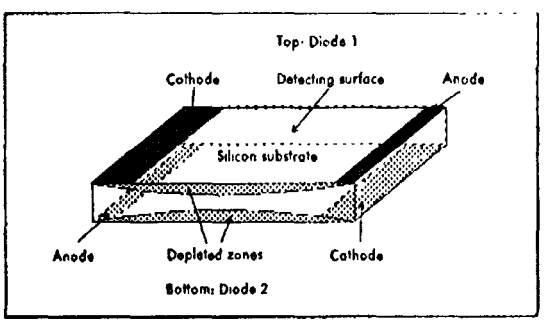
The objectives for the next period are devoted to the studies of the main dosimetric characteristics such as :

- determination of detection threshold in terms of dose equivalent and rate,
- energy response for mono-energetic neutrons,
- global response in realistic fields,
- transition to the single diode system.

IV - Progress achieved including publications

Principle of the double diode detector :

To avoid any discrepancy between the two diodes, we designed a double diode on the same silicon substract located in the opposite side. The substrate thickness is of about 250 μm. In these conditions, the two depleted zones have the same depth at each point of the surface. For one to another point of the surface, the depth can vary within 30 % of the mean value. This situation presents a double interest, that is to say, both the same sensitivity and the same background noise. The following figure shows the schematic diagram of the double diode.



Advantages and disadvantages

- Better symmetry between the two diodes,
- Background noises very similar,
- Different direct scattering and back scaterring for photons.

References :

J. BARTHE et al.
Personal neutron diode dosemeter -
Rad. Prot. Dos., 47 1/4 (1993), pp. 397-399.
J. BARTHE et al.
Etude de compteurs miniatures..., -
Proc. IRPA8 Vol. 1 Montréal (1992), pp.479-482.
J. BARTHE et al.
New devices for individual neutron dosemeters -
Int. Workshop on Individual. Monitoring. May- 5-7, 1993 Villigen, Switzerland.

Head of project 4 : Prof. FERNANDEZ MORENO

II - Objectives for the reporting period

The main objective is the stage of an investigator at the Limoges University, in order to study electronic methods.

III - Objectives for the next period

The objectives for the next period are devoted to experimental cond theoritical results sach as :

* Implementation of a diode based microcomputer pulse analysis system.
* Monte Carlo simulation of the neutron interaction in the diode.
* Calculation of the γ contribution with the EGS 4 code.

IV - Progress achieved including publications

Stage of an investigator at the Limoges University :

Mr. Tayeb Bouassoule spent three months in studying the electronic neutron dosemeter existing at the Limoges University in order to implement the system in our laboratory.

This work has been divided into three parts :

1) The first part consisted in the bibliographic study of the physical concepts related to passivated ion-implanted silicon diodes.

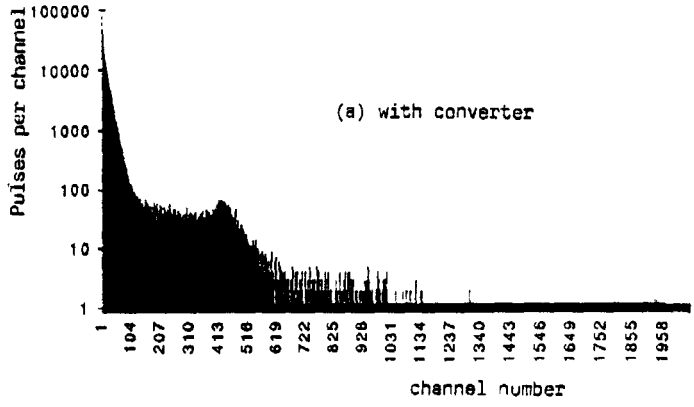
2) The electronic components of the detection system have been set up and calibrated.

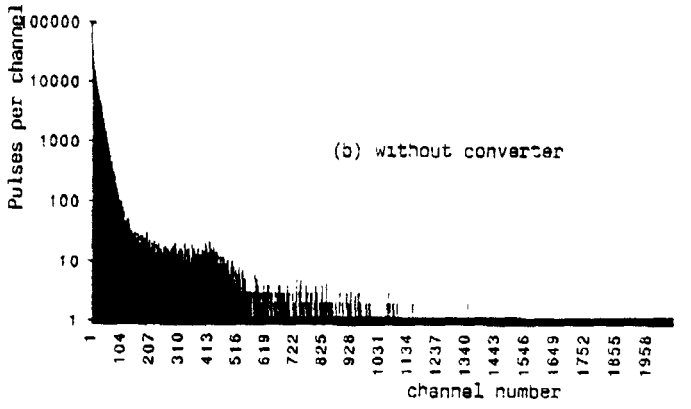
The calibration has been carried out using the Camberra AccuSpec Nal code with the aid of two alpha particle sources : ^{233}U and ^{241}Am.. The calibration result was :

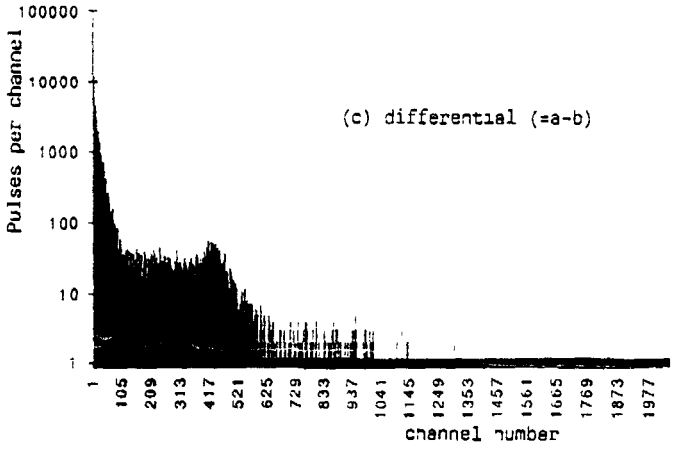
$$E(keV) = 0,5810 + \text{channel number} \times 5,419.$$

3) The irradiation of two diodes during one hour with Am-Be neutron source, one of them covered with a polyethylene converter. The differential method used for analysis needs the subtraction of the results obtained from the diode without converter from the results obtained from the diode with converter. According to the theoretical predictions, these results correspond to the protons emerging from the converter with an energy spectrum showing a maximum at an energy equal to half the maximum neutron energy, as displayed in the figure.

We have found that the background levels obtained for the two diodes are different. In addition, some high energy pulses, of unknown origin, are present.







Diode response to neutron irradiation

Head of project 5 : Prof. CURZIO

II - Objectives for the reporting period

The main objective was the investigation of the response of super-heated drop (bubble) detectors as a combined function of neutron energy and of their degree of superheat. The purpose was twofold : first, we aimed at the accurate definition of the effects of environmental temperature changes on the response of the detector ; second, we wanted to optimise the performace of the detector when used as a dosemeter-i.e., define the degree of super-heat corresponding to the best dose equivalent response.

III - Objectives for the next period

Measurements on prototype device will be performed with n-γ beams, and comparisons realized through joint irradiations in the framework of the contract.

The fluence and dose equivalent response of the detector to monoenergetic epithermal and intermediate neutrons - which are not experimentally available - will be studied by means of Monte-Carlo simulations.

Next, super-heated materials with a threshold response suitable for radiation protection spectrometry will be charaterized and appropriate unfolding techniques will be studied.

IV - Progress achieved including publications

The detectors we employed in the energy response determinations are vial containing 4 cm^3 of a sensitive composition-i.e., a suspension of about 20000 super-heated halocarbon-12 drops of 100 μm diameter dispersed in an aqueous gel.

Bubbles were counted acoustically, detecting the pressure wave that accompanies each vaporisation event. For this purpose, a meter similar to the one described by Apfel and Roy[1] was employed. Temperature regulation of the detectors was achieved by means of thin, flexible etched-foil heating elements wrapped around the vials along with a ribbon of Platinum resistance-temperature-sensor. Heaters and thermometer were surrounded by a light-weight latex-foam thermal insulator and were operated by a time proportioning controller (Figure 1).

The experimental investigations were mainly carried out at the PTB, in Braunschweig. For the free-in-air response determinations, monoenergetic neutron fields in the thermal to 19 MeV energy range were produced at the 1 MW research and measurement reactor (FMRB) and at the 4 MV Van de Graaff accelerator. At the FMRB, collimated filtered neutron beams[2] of 144 keV and thermal neutrons-the latter using a Cadmium filter

difference method to eliminate contaminating fast neutrons-were employed for this study.

The beam cross sections are large enough to cover the detector vial entirely-diameters are 13,3 and 4.2 cm, respectively, at half maximum flux density. Irradiations with monoenergetic neutron fields of 0.07, 0.144, 0.25, 0.57, 1.2, 2.5, 4.0, 5.0, 14.8, and 19 MeV were carried out in the low-scatter hall of the PTB Van de Graaff accelerator[3].

Tests with higher energy neutrons were performed at the 590 MeV proton accelerator of the Paul Scherrer Institut (PSI)[4], in Villigen (Switzerland), where the DCMN informally participated in the 1992 Eurados intercomparison of nuclear-track detectors.

The free-in-air fluence response of the superheated-drop detector as a combined function of energy and temperature is illustrated in Figure 2. Plot symbols include the error bars ; relative standard deviations are in the range 2-8 %, against an expected 3-4 % based on Poisson statistics. At 25°C, the two high energy points from the PSI tests are reported : average values of 25 and 30 MeV are used to represent the observed response to the whole energy range. Error bars along the energy axis approximate the width of the two broad neutron spectra.

The predominant effect on the response as the degree of superheat increases is a threshold shift towards lower energies. This parallels the decrease in the minimum energy deposition which is necessary to nucleate bubble formation.

These investigations show that when the halocarbon-12 based detector is kept at a constant temperature of 32°C, its ambient dose equivalent response to thermal and fast neutrons falls within a factor 1.5 of 3.5 bubbles per μSv (Figure 3). This meets the recommendations on the accuracy of measurements set by the International Commission on Radiological Protection, as well as those on the minimum dose to be detected by area monitors[5].

1 - Apfel, R.E. and Roy, S.C. *Instrument to Detect Vapor Nucleation of Superheated Drops.* Rev. Sci. Instrum. 54(10) 1397-1400 (1983).

2 - Alberts, W.G. and Dietze, E. *Filtered Neutron Beams at the FMRB - Review and Current Status.* Report PTB-FMRB-112 (PTB, Braunschweig) (1987).

3 - Brede, H.J., Cosak, M., Dietze, G., Gumpert, H., Guldbakke, S., Jahr, R., Kutscha, M., Schegel-Bickmann, D., Schölermann, H., *The Braunschweig Accelerator Facility for Fast Neutron Research, 1 : Building, Design and Accelerators.* Nucl. Instr. Methods. 169 349-358 (1980).

4 - Schuhmacher, H. and Alberts, W.G. *Reference Neutron Fields with Energies up to 70 MeV for the Calibration of Radiation Protection Instruments.* Radiat. Prot. Dosim., 42 287-290 (1992).

5 - Portal, G., and Dietze, G. *Implications of New ICRP and ICRU Recommendations for Neutron Dosimetry.* Radiat. Prot. Dosim. 44(1.4) 165-170 (1992).

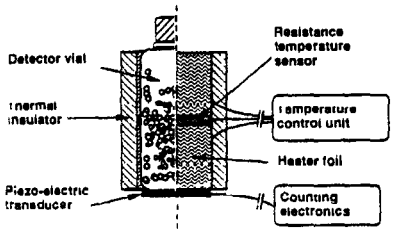


Figure 1. Superheated drop detector probe for the determination of the response as a combined function of temperature and neutron energy.

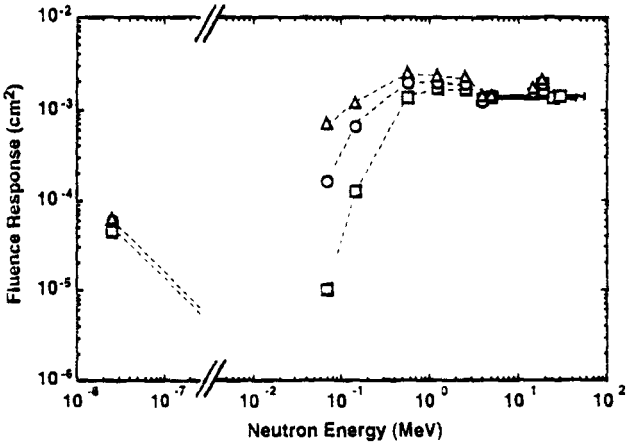


Figure 2. Fluence response of the superheated drop detector as a function of neutron energy, measured at 25 (□), 30 (Δ), and 35 (O) degrees Celsius. Dashed lines are eye-guides only.

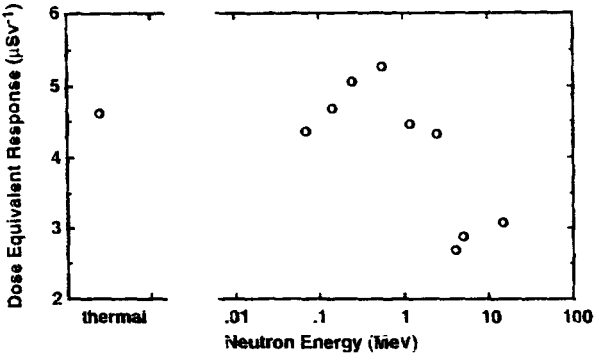


Figure 3. Ambient dose equivalent of SDD as a function of neutron energy, measured at 32°C. (Fluence to dose equivalent conversion factors as in reference (5)).

Progress Report

Contract: **FI3P-CT920048** **Sector: A14**

Title: Assessment of internal dose from plutonium and other radionuclides using stable isotope tracer techniques in man.

1)	Roth	GSF Frankfurt
2)	Molho	Università degli Studi di Milano
3)	Taylor	University of Wales, Cardiff
4)	McAughey	AEA Technology Harwell Laboratory

I. Summary of Project Global Objectives and Achievements

A) Global objectives of the project

This coordinated project continues the work carried out under contract BI7-0029 "Assessment of internal dose from radionuclides using stable isotope tracer techniques in man", CEC Radiation Protection Research Programme 1990 - 1992.

The global objective of this project is to provide human biokinetic data for some radionuclides of relevance in radiation protection by use of stable isotopes as tracers in metabolic investigations. The transfer of radionuclides into the human body via the food chain shall be investigated by experimental studies in man, with particular attention to the reliability and variability of transfer parameters under realistic conditions. Additional investigations on the internal distribution and excretion patterns shall improve the metabolic and dosimetric models and consequently the dose assessments of internal exposure.

The metabolic behaviour of some radionuclides can be studied by substituting the radioactive isotopes by stable isotopes of the same element as tracers. For plutonium and other highly toxic radioelements, where no stable isotopes exist, it shall be evaluated if stable analogues can be used in metabolic studies. The use of selected elements of the lanthanide series as surrogates for actinides in stable element tracer studies in human volunteers should provide valuable information which is not otherwise obtainable by planned systematic investigations.

The goals of this project require the close collaboration of several laboratories to contribute with their special expertise and experience in the different aspects involved. The work of each of the laboratories depends largely on the results obtained by the other ones. The cooperation among the participating laboratories was succesfully established during the previous contract. Several analytical techniques for the measurement of the stable isotopes in tissue and excretion samples have already been developed and optimised for a number of elements. Based on these results, these experiences will guide the development of analytical techniques for the measurement of hafnium, barium and other elements in samples of

biological materials. Whereas barium isotopes will be measured predominantly by mass spectrometric methods, the analytical techniques for the quantitative measurement of stable hafnium isotopes in biological samples must be developed and optimised. Exchange of samples among the laboratories for measurements will help in defining the most suitable methods.

B Summary of achievements

B1 Molybdenum

Analytical methods for the determination of molybdenum and specific molybdenum isotopes in solutions and biological materials were successfully developed and applied (Project 1 and Project 2). The occurence of molybdenum in various vegetables was evaluated and found to be in broad agreement with published data (Project 1). The feasibility of stable molybdenum isotopes for biokinetic investigations was verified by animal experiments (Project 2). For investigations in humans a protocol was developed for the simultaneous administration of an oral and an intravenous tracer.

Oral administration of stable Mo isotopes to human volunteers revealed f_1 - values that are generally lower than the recommended ICRP values of 0.8 (Project 2). Two stable Mo isotopes were administered simultaneously (orally and intravenously) to a healthy volunteer to evaluate internal Mo distribution. Blood half time was 1 hour, which is considerably shorter than the adopted ICRP value of 6 hours (Project 2).

B2 Zirconium

Proton nuclear activation analysis (PNA) appears to be a suitable analytical method for determination of stable zirconium isotopes ^{90}Zr and ^{96}Zr (Project 2). The availability of two stable Zr isotopes of low natural abundances is a major advantage for the planned double isotope studies in humans. Minimum detectable concentrations in plasma by PNA are 2 ng/ml for both ^{90}Zr and ^{96}Zr (Project 2). It can be expected that the amount of stable zirconium used in tracer investigations in man does not perturb significantly the metabolic processes to be evaluated.

B3 Strontium and Calcium

Continuing previous investigations on Sr uptake in man, ^{43}Ca and ^{84}Sr were administered to a neonate (Project 4). Neonates form a critical group in the assessment of ^{90}Sr dosimetry, as they have a high rate of Ca uptake and Sr is incorporated with Ca into bone. Preliminary results from this study indicate that Ca and Sr excretion in urine after oral administration follow a similar pattern in newborns. These measurements form a pilot study, the results of which are being used to design the next phase which will involve a larger group of neonates.

Aliquots of the biological samples have been circulated to the other laboratories in order to evaluate the reliability of the analytical methods applied.

B4 Barium and Neodymium

Studies of fractional gut absorption were continued in a group of 4 male and 4 female volunteers (Project 4). Simultaneous oral and intravenous administrations of Ba and Nd were given. These experiments have been designed to demonstrate whether fractional gut absorption is enhanced following a fast, and whether simple ligands present in the diet, such as citrate, can further enhance uptake.

For Ba, as for earlier Sr data, it is shown that fractional gut uptake is significantly higher than the adopted ICRP value of 0.1 when subjects have fasted prior to administration. Whole body retention of injected Ba after 7 days showed large variability between individuals. For Nd, fractional gut uptake is also enhanced in individuals who have fasted prior to ingestion, with a number of the fasted uptake values exceeding the adopted ICRP value of 3×10^{-4}. Excretion of Nd was principally via urine with whole body retention found to be 0.94 ± 0.03 after 7 days (Project 4).

B5 Lanthanides (as analogues for actinides)

The chemical similarities between the elements of the lanthanide and actinide series suggest that carefully selected lanthanide isotopes could be used as surrogates for highly toxic actinides.

Europium and Gadolinium (as surrogates for Americium and Curium)

Computer speciation methods were used to model chemical speciation profiles for the interaction of the lanthanides, europium and gadolinium, and the actinide metals americium and curium, with the five biologically important low molecular mass ligands citrate, lactate, oxalate, tartrate and acetate in order to assess the suitability of europium and gadolinium for use as surrogates for americium and curium for biokinetic studies in humans (Project 3). The speciation behaviour of each radionuclide in the presence of each ligand was modelled using the computer programme MINTEQA2.

For americium and europium, comparison of the profiles generally showed very good agreement for most of the ligands studied. In contrast, with gadolinium and curium the speciation profiles were often divergent. The speciation behaviour of Cm appears to be significantly different from that of Eu, Gd or Am, largely due to its tendency to undergo early hydrolysis (Project 3).

The conclusion from this work is that Eu can be used as an analogue for Am, but Gd may be a better choice. However, the differences between Cm^{3+} and Gd^{3+} indicate that the latter cannot be used as a Cm analogue. Further work is needed to identify a suitable analogue for Cm.

Hafnium (as surrogate for Plutonium)

Results obtained under the previous contract indicate that hafnium may serve as surrogate for plutonium in biokinetic investigations. A comparative study of the chemical speciation behaviour of Hf and Pu will be part of this contract carried out during the next period to assess the suitability of Hf also for human studies (Project 3).

Analytical methods for the measurement of stable Hf isotopes in biological samples are currently being optimized. Preliminary results of PNA measurements indicate that the sensitivity for Hf in plasma is of the order of 10 ng/ml for ^{176}Hf and ^{178}Hf (Project 2).

After the oral administration of radioactive ^{175}Hf and ^{237}Pu to rats there seem to be some differences in the organ distribution of the two elements. Similar studies involving injection and administration of stable isotopes of Hf and Nd are currently being performed. Furthermore, preparations are now in progress for a study of Hf uptake by volunteers (Project 4).

C) Conclusions

The use of stable isotopes in tracer investigations can provide valuable human metabolic data which is not otherwise obtainable, thus improving the comparatively poor data base for some relevant radionuclides. The results of this co-ordinated project obtained so far are encouraging. They indicate that for a number of elements the currently adopted dosimetric factors must be revised, whereas others could be verified.

Applying these techniques it will be possible to study the variability of the biokinetic parameters more systematically, e.g. uptake from various contaminated foodstuffs and factors affecting it.

A close and fruitful collaboration has been established among the participating laboratories from Germany, the United Kingdom and Italy. To a large extent, it is this cooperation that enabled the progress achieved which in turn provides the basis for the development and success of these studies.

Head of Project 1: Dr. Roth

II. Objectives for the reporting period:

1. Development of analytical techniques for the measurement of molybdenum concentrations in biological materials and for the determination of stable molybdenum isotopes by means of mass spectrometry

2. To perform experimental studies on the intestinal uptake of molybdenum in humans

3. Exchange of biological samples for the optimization of analytical methods and of samples from in vivo studies for comparison of results between Milan, Harwell, and Frankfurt

Objectives for the next period:

1. Investigations of molybdenum transfer into humans via the food chain

2. Development of countermeasures to reduce uptake of radioisotopes of molybdenum from food into humans in emergency situations

3. Development of analytical techniques for the determination of hafnium concentrations in biologial materials and for the measurements of stable hafnium isotopes by means of mass spectrometry

4. To prove and modify the metabolic and dosimetric models for the elements investigated in these projects, and to perform new dose calculations for the incorporation of the respective radionuclides.

III. Progress achieved

Analytical

Molybdenum is an essential trace element in humans, although Mo concentrations are reported to be low in human blood plasma. Due to severe matrix effects direct measurements of Mo concentrations in human blood plasma and urine by means of atomic absorption spectrometry proved not to be possible. Consequently, different methods for the extraction of Mo from biological samples were tested. Nearly quantitative extraction of Mo was achieved with liquid-liquid extraction of Mo from wet ashed samples applying di(2ethylhexyl)phosphoric acid (HDEHP). Up to 1 ml of blood plasma or urine were wet ashed under pressure with 0.8 ml of concentrated nitric acid. Mo is than extracted in 2 ml of 0.03molar HDEHP solution. The organic solution can be measured directly by means of atomic absorption spectrometry, applying the standard addition technique. In a group of 18

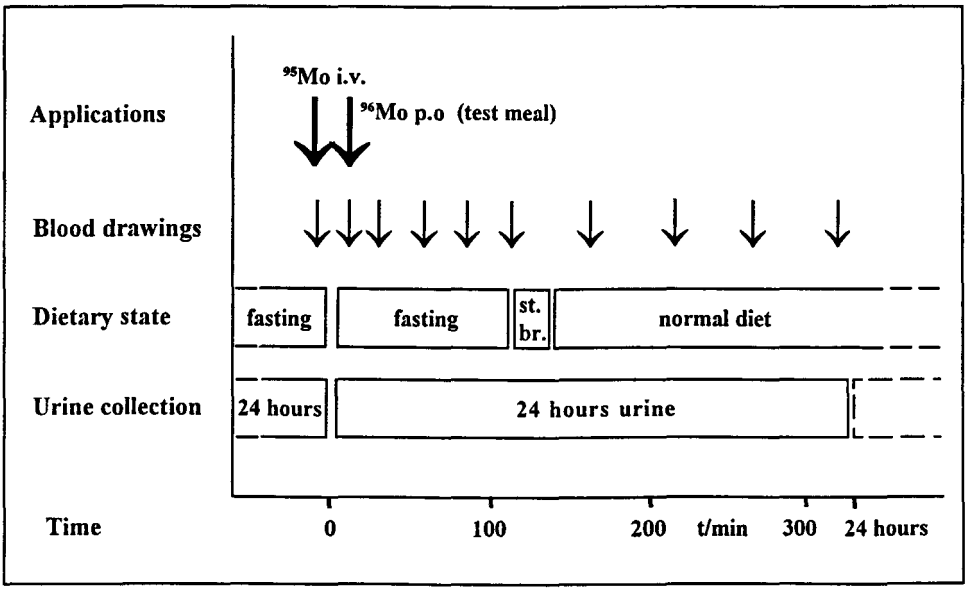
healthy male volunteers (age: 45 - 62) a mean Mo concentration in plasma of 4 μg/l (range: 0.3 to 12 μg/l) and a mean Mo excretion in 24 hours urine of 50 μg(range: 1 - 129 μg) was found. These values are in accordance with the limited data found in literature.

For the mass spectrometric measurements of stable Mo isotopes in biological materials a further preparation step is necessary to reduce organic substances. An additional extraction of Mo in ammonia and further wet ashing seems to be sufficient. Up to now the procedure for the measurements of Mo isotope ratios by means of thermal ionization mass spectrometry in aqueous solutions was developed. Because of the high ionization potential of Mo the measurement of ions at the respective mass numbers in the positive spectrum requires a high amount of Mo (40 μg). Using the detection of negative MoO_3^- ions and the very sensitive ion counting technique, isotope ratios in amounts of about 40 ng of Mo can be measured with a reproducibility of less than 1% for all isotope ratios. This was achieved by a special preparation and heating technique. To enhance ionization of molybdenum, 1 μg of calcium was loaded onto the ionization filament, the molybdenum solution was prepared on the evaporation filament. The filament currents were increased in six alternating heating steps. For the calculation of the intensities of single Mo masses a correction for the different oxygen isotopes has to be performed.

Human studies

For the assessment of intestinal absorption of Mo in healthy human subjects a protocol was developed applying the double isotope method and the evaluation of fractional intestinal absorption by the convolution integral technique (Figure 1).

Figure 1: Scheme of study protocol for evaluation of f_1 - values in humans



To employ this technique subjects received after an overnight fast the test dose / test meal labelled by one stable Mo isotope (e.g. ^{96}Mo) and at the same time an intravenous injection of another highly enriched stable Mo isotope (^{95}Mo) in sterile saline. Blood samples each of 10 ml were drawn before administration and 15, 30, 60, 90, 120, 180, 240, 300 min, and 24 hours after administration. Additionally urine was collected at least for 24 hours. These studies on intestinal Mo absorption were perfomed in healthy subjects, applying aqueous solutions of 500 μg and 1000 μg Mo respectively dissolved in hydrochloric acid. Analysis of these biological samples by mass spectrometry is currently being in progress.

Aliquots of these samples were also transferred to the Milan group for analysis of ^{95}Mo and ^{96}Mo contents by means of proton nuclear activation analysis (see Project 2). This will allow to evaluate intestinal Mo uptake in humans (f_1 - values) by two independent methods, thus establishing not only the reproducibility of the methods but also their accuracy and reliability. This is only possible due to the close cooperation of the two institutes in Milan and Frankfurt.

For future human studies of intestinal Mo uptake from foodstuffs, the Mo concentrations in a series of vegetables were evaluated (Table 1). The data obtained are in general agreement with values published in the literature.

Table 1: Mo concentrations in various vegetables

Vegetable	Mo concentration (ng/g)
Stock beet	150 ± 30
French bean	150 ± 40
Tomatoe	90 ± 35
Spinach	75 ± 20
Fennel	50 ± 15
Broad beans	45 ± 20
Basil	40 ± 10
Mushrooms	40 ± 20
Zucchini	35 ± 15
Radish	30 ± 10
Cucumber	30 ± 10

Since in many procedures only the reproducibility of the results can be checked and not the accuracy, it is of substantial significance especially in such scientific investigations to develop a measure of accuracy by evaluation of results applying independent methods. Therefore, samples of ashed faeces which were already investigated for its enriched ^{43}Ca content by ICPMS in Harwell (see Project 4) were prepared for thermal ionization MS, which will be carried out in the next period.

Publications

1.) M.C.Cantone, D. de Bartolo, N.Molho, L.Pirola, G.Gambarini, Ch.Hansen, P.Roth, E.Werner. Molybdenum metabolism studied by means of stable tracers. Med. Phys. 19, 439-444 (1992).

2.) M.C.Cantone, D. de Bartolo, G.Gambarini, A.Giussani, N.Molho, L.Pirola, Ch.Hansen, P.Roth, E.Werner. Intestinal absorption of tellurium studied with stable tracers. J. Radioanal. Nucl. Chem. 170, 2, 433-442 (1993).

3.) M.C.Cantone, D. de Bartolo, N.Molho, L.Pirola, G.Gambarini, Ch.Hansen, P.Roth, E.Werner. Response to a single oral test of molybdenum stable isotopes for absorption studies in humans. Physiol. Meas. 14, 217-225 (1993).

4.) N.Molho, M.C.Cantone, D. de Bartolo, G.Gambarini, A.Giussani, L.Pirola, Ch.Hansen, P.Roth, E.Werner. Use of cyclotron for trace element analysis in biological samples and related metabolism studies. Nucl. Instrum. Meth. B79, 560-563 (1993).

5.) M.C.Cantone, D. de Bartolo, N.Molho, L.Pirola, G.Gambarini, Ch.Hansen, P.Roth, E.Werner. Use of stable Ru in assessing Ru clearance: experiments in animals. Phys. Med. in press (1993).

Head of project 2: Prof. Molho (Dr. Cantone)

II. Objectives for the reporting period

The main objectives for our group were:

- the development of activation analytical methods for the quantitative determination of stable isotopes of hafnium and zirconium in biological samples;

- the evaluation of intestinal uptake of molybdenum in man by means of stable isotopes.

Objectives for the next period

- the optimization of activation analytical methods for the quantitative determination of stable isotopes of hafnium in biological samples;

- the study of intestinal absorption of zirconium in animals by means of stable isotopes;

- investigations of intestinal uptake of molybdenum in humans by means of stable isotopes in consideration of the possible reduction of Mo absorption;

III. Progress achieved

A) Optimization for Zr and Hf stable isotope determination

A preliminary study, on the basis of the natural isotopic composition of Zr, of the possible nuclear reactions induced by protons on the Zr stable isotopes and of the decay characteristics of the radioactive isotopes obtained from such reactions, shows that 90-Zr and 96-Zr are the most suitable isotopes to be used as tracers. In fact, via (p,n)-reactions, the produced radioactive 90-Nb and 96-Nb are suitable for PNA analysis from the point of view either of energies and intensities of the gamma decay transitions (90-Nb: 1129 keV, 90% ; 96-Nb:779 keV, 96%) and of the respective half lives (14.6 h; 23.5 h) sufficiently long to allow an off line detection and a significant reduction of the biological matrix background.

The availability of two Zr isotopes simultaneously detectable is essential in view of the determination of intestinal absorption by means of the double tracer technique.

In order to optimize the conditions of measurement, the proton energy corresponding to the maximum yield of the chosen reactions was determined. To this aim we prepared a sandwich containing 8 high purity 0.1 mm thick Zr foils separated by 0.1 mm aluminum foils and the stack was irradiated with a 18.5 MeV energy proton beam. The analysis of the gamma-ray spectra collected from each Zr foil, using the same geometry and the same collecting time, allows us to estimate that the maximum yields for 90-Nb and 96-Nb are obtained for proton energies of 14 MeV and 10 MeV.

Measurements for the evaluation of detection limits of PNA for 90-Zr and 96-Zr in plasma samples were performed. By considering as minimum detectable quantity the one corresponding

to a peak area equal to three times the square root of the subtended background we obtained as minimum detectable concentration in plasma the value of 2 ng/ml for 90-Zr and 96-Zr respectively.

We followed the same procedure for the optimization of PNA in view of the simultaneous determination of two stable isotopes of Hf in plasma.

In principle, from the analysis of half-lives and gamma-ray energies of the radioactive Ta isotopes produced via (p,n)-reactions from the stable isotopes of Hf we deduced the possibility of determining three isotopes of Hf, which are: 176-Hf, 177-Hf and 178-Hf. Due to the fact that, in case of contiguous isotopes to be simultaneously determined, interference reactions, producing the same radioisotopes of interest, cannot be avoided, we prefered to devote our attention to 176-Hf and 178-Hf.

For Hf, at this point of the work, we have only a very rough indication of the sensitivity of PNA in plasma, that it is of the order of 10 ng/ml for each isotope, as determined by irradiating plasma samples doped with 50 ng and 1 μg of natural Hf.

B) Evaluation of intestinal uptake of molybdenum in man by means of stable isotopes.

In a pilot study we optimized PNA for the simultaneous determination of two stable isotopes of molybdenum (95-Mo and 96-Mo) and we performed a study of the intestinal absorption of Mo in rabbits (For details see Publication 1). Thereafter, two volunteer subjects were given orally enriched solutions of 95-Mo and 96-Mo containing 1 mg of each isotope in aqueous solution. Fractional intestinal absorption (f_1 - values) were 0.53 and 0.57 respectively (For details see Publication 2).

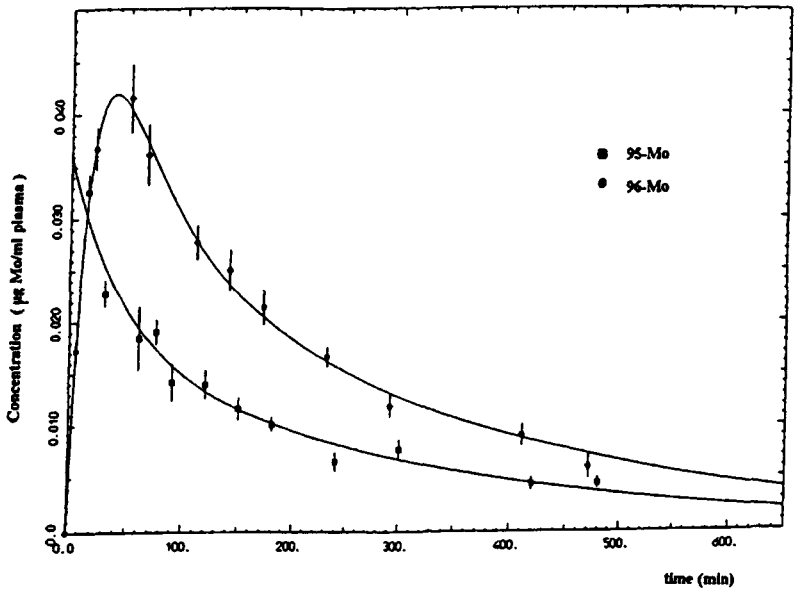


Fig.1: Concentration of the injected tracer 95-Mo and of the ingested tracer 96-Mo, as a function of time

After this study we performed in a healthy volunteer a complete investigation on molybdenum absorption by means of the double tracer technique. The subject (51 years, 85 kg, 3860 ml plasma volume) was injected intravenously 442 μg of 95-Mo and was orally given 940 μg of 96-Mo. Twelve venous blood samples were withdrawn within 480 min after application. The concentration of the tracers in plasma samples, determined by PNA, are shown in Fig.1, as a function of time.

By convolution integral technique, the fractional intestinal absorption was calculated to be 0.27 ± 0.03. For more details, the data have also been fitted acoording to the compartmental model shown in Fig.2. In Tab.1 the obtained values for the fractional transfer coefficients and the fractional intestinal absorption are reported.

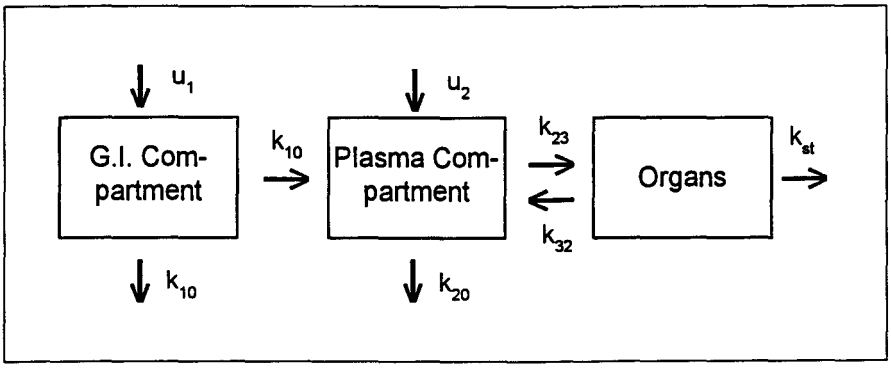


Fig.2: Compartmental model for the metabolism of molybdenum

Table 1: Fractional transfer coefficients for the molybdenum model and fractional intestinal absorption of molybdenum in a healthy human volunteer

Fractional transfer coefficients (min^{-1})	
k_{10}	= 3.9 E-2
k_{12}	= 1.4 E-2
k_{23}	= 7.2 E-3
k_{32}	= 1.3 E-3
k_{20}	= 5.8 E-3
Fractional intestinal absorption	
f_1	= 0.27 ± 0.03

Publications

1.) M.C.Cantone, D. de Bartolo, N.Molho, L.Pirola, G.Gambarini, Ch.Hansen, P.Roth, E.Werner. Molybdenum metabolism studied by means of stable tracers. Med. Phys. 19, 439-444 (1992).

2.) M.C.Cantone, D. de Bartolo, G.Gambarini, A.Giussani, N.Molho, L.Pirola, Ch.Hansen, P.Roth, E.Werner. Intestinal absorption of tellurium studied with stable tracers. J. Radioanal. Nucl. Chem. 170, 2, 433-442 (1993).

3.) M.C.Cantone, D. de Bartolo, N.Molho, L.Pirola, G.Gambarini, Ch.Hansen, P.Roth, E.Werner. Response to a single oral test of molybdenum stable isotopes for absorption studies in humans. Physiol. Meas. 14, 217-225 (1993).

4.) N.Molho, M.C.Cantone, D. de Bartolo, G.Gambarini, A.Giussani, L.Pirola, Ch.Hansen, P.Roth, E.Werner. Use of cyclotron for trace element analysis in biological samples and related metabolism studies. Nucl. Instrum. Meth. B79, 560-563 (1993).

5.) M.C.Cantone, D. de Bartolo, N.Molho, L.Pirola, G.Gambarini, Ch.Hansen, P.Roth, E.Werner. Use of stable Ru in assessing Ru clearance: experiments in animals. Phys. Med. in press (1993).

Head of Project 3: Prof. Taylor

II. Objectives for the reporting period:

a. The objectives for this reporting period were to use computer methods of chemical speciation analysis to compare the behaviour of the lanthanide metals, europium and gadolinium, and the actinide metals americium and curium, in the presence of the biologically important ligands citrate, tartrate, lactate, acetate and oxalate, in order to assess the suitability of europium and gadolinium for use as surrogates for americium and curium for biokinetic studies in humans.

Objectives for the next period:

a. The work for the next year will be directed to the further examination of the apparent differences in the speciation behaviour of curium in comparison with americium, europium and curium.

b. To carry out a similar comparative study of the chemical speciation behaviour of hafnium and plutonium with citrate, and other ligands, in order to further assess the suitability of hafnium as a surrogate for plutonium for human studies. To further examine lanthanide and actinide interactions with the iron-transport protein transferrin and to refine the estimates of the formation constants for the metal-protein complexes.

c. To begin the comparison of the speciation behaviour of lanthanides and actinides with published data on their biodistribution and biokinetics of these elements.

III. Progress achieved

The chemical similarities between the elements of the lanthanide and actinide series suggest that carefully selected lanthanide isotopes could be used as surrogates for the highly toxic actinides plutonium, americium and curium for biokinetic studies in humans.

Computer speciation methods have been used to model chemical speciation profiles for the interactions of the lanthanides, europium and gadolinium with the five biologically important low molecular mass ligands citrate, lactate, oxalate, tartrate and acetate in order to assess their suitability as analogues for the actinides americium and curium.

Chemical speciation describes the state in which metals are present in solution, i.e. their valence states, their presence as free aquated ions or as metal-ligand complexes. In computer speciation analysis the parameters included in the input data for the computer speciation programme are of critical importance, these must include information on the mass and charge and concentrations of the component metals and ligands, as well as thermodynamic formation constants for all the possible interactions of the components.

The computer programme used was MINTEQA2, a primarily geochemical equilibrium speciation model which is capable of calculating the chemical equilibrium distribution of

species in dilute aqueous systems, once total component concentrations and the relevant formation constants are known. MINTEQA2 includes in its own database many hundreds of thermodynamic formation constants, and ligand protonation constants, but none were relevant to this work. Thus an extensive literature search was a vital preliminary task in order to compile our own MINTEQA2 database. Only constants measured at temperatures of 20°C or 25°C were used and all values were corrected to zero ionic strength, I = 0, using the Davies Equation [Davies, C. W., Journal of the Chemical Society, 1939, 2093.]. Wherever possible all data for a particular species were taken from the same author to minimise errors.

The speciation behaviour of each radionuclide in the presence of each ligand was modelled using MINTEQA2. For initial runs, the total concentration for both metal and ligand was set at 0.1 M and the system allowed to equilibrate from pH 2 to pH 10. This enabled the speciation profile to be presented graphically as a plot of species concentration against pH, and clearly showed the species distribution at physiological pH 7.4.

The number of different species which can be assumed to be present at equilibrium is limited by the available experimental data. Many data are available for Am(III), but far fewer for its proposed analogue, Eu(III). For example the data available for Am^{3+} and $citrate^{3-}$ included $AmOH^{2+}$, $Am(OH)_2^+$, $Am(OH)_3$, AmCit, $Am(Cit)_2^{3-}$, $AmHCit^+$ and $AmCitOH^-$, allowing the possibility of seven different species to be formed when the system equilibrates. In contrast the data for Eu^{3+} and Cit^{3-} included only $EuOH^{2+}$ and EuCit, so the formation of only two species could be considered. This posed a problem with respect to obtaining a fair comparison of the behaviour of Am and Eu in the presence of citrate, since only "like" species should be compared. To remedy this the initial run was done including all the data for Am to give an accurate and informative profile of its behaviour. Then, in order to compare the profile with Eu, a run was carried out including only $AmOH^{2+}$ and AmCit since these were the only species available for Eu. In most cases this meant omission of the higher complexes and higher hydroxides, e.g. $M(L)_3$ and also the omission of the protonated and higher hydroxy complexes, e.g. $ML(OH)_x$. The results for these "like with like" speciation runs are shown in Table 1, which records only the percentage of the dominant species present when the system equilibrates at pH 7.4.

The simplified Am speciation model and the Eu model for citrate showed that at pH 7.4 100% of the total metal in the system is present as the monocitrate complex for both metals. This indicates very good agreement. However, when the entire speciation graph for all the Am species was considered $AmCitOH^-$ appeared as the dominant species, accounting for 95% of the total Am. This is quite different from the simple Eu model and might suggest that the two metals behaved differently. However, since in the simple systems, both metals showed the same speciation, it has been assumed that the Eu profile would in fact be similar to that for Am if the formation constants for the other Eu species had been available.

For Am and Eu, comparison of the "like with like" profiles generally showed very good agreement for most of the ligands studied, for example with citrate 100% complexing of both metals was achieved at about pH 4. The acetate profiles showed a similar pattern, but Eu hydrolysed less rapidly than Am, as demonstrated by the smaller percentage of Am acetate in Table 1. The only noticeably different profiles were seen with oxalate and this is echoed by the values in Table 1, the monooxalate was dominant over most of the considered pH range, but at pH 7.4 this species accounted for 76% of the Eu(III), but only 52% of the Am(III)

Table 1. The percentage of the dominant species present at physiological pH 7.4

Metal Ligand	Eu(III)	Am(III)	Gd(III)	Cm(III)
$Citrate^{-3}$	EuCit 100%	AmCit 100%	GdCit 100%	CmCit 100%
$Lactate^{-}$	$EuLact^{+2}$ 90%	$AmLact^{+2}$ 90%	$GdLact^{+2}$, 80%	$CmOH^{+2}$ 83%
$Oxalate^{-2}$	$EuOx^{+}$ 95%	$AmOx^{+}$ 45%	$GdOx^{+}$ 90%	$Cm(Ox)_2/CmOH^{+}$ 40%
$Tartrate^{-2}$	$EuTart^{+}$ 95%	$AmTart^{+}$ 95%	$GdTart^{+}$ 95%	No data
$Acetate^{-}$	$EuAc^{+2}$ 50%	$AmAc^{+2}$ 40%	$GdAc^{+2}$ 38%	$Cm(OH)_2^{+}$ 100%

In contrast, with Gd(III) and Cm(III) only the speciation profiles were often divergent. For both lactate and acetate the Cm and Gd profiles were similar up to about pH 4, but thereafter the proportions of the various species began to diverge with the $CmLact^{+2}$ species beginning to dissociate at about pH 5 while the $GdLact^{+2}$ species remained stable up to pH 7. Also, in the presence of lactate or citrate, Cm begins to hydrolyse at pH 3-4 so that by pH 8 $CmOH^{+2}$ is the predominant species, in contrast Gd does not hydrolyse appreciably below pH 7. The lactate profiles for all four elements are shown in Figure 1, from which it can be seen that the speciation behaviour of Cm is significantly different from that of Eu, Gd or Am, largely due to its tendency to undergo early hydrolysis.

No data are available for Cm and tartrate, so the Gd tartrate profile was compared with Am tartrate instead. The profiles were very similar and Gd appeared to be closer to Am than Eu; this was also observed with lactate and acetate Since in blood plasma the ligand concentration is about 10^{-5} M, the Am, Gd and Cm citrate systems were remodelled at this total concentration. The effect of reducing the concentration was to increase hydroxide formation at lower pH values, and to increase the percentages of the free ion at higher pH. The Gd citrate system was also studied at a metal.ligand ratio of 1:5, a situation closer to that found in plasma, this increased the formation of $Gd(Cit)_2^{-3}$, which at pH 7.4, reduced the total percentage of the dominant hydroxy-citrate species

The conclusion from this work is that Eu can be used as an analogue for Am, but Gd may be a better choice. However, the differences between Cm^{3+} and Gd^{3+} indicate that the latter cannot be used as a Cm analogue. Further work is needed to identify a suitable analogue for Cm.

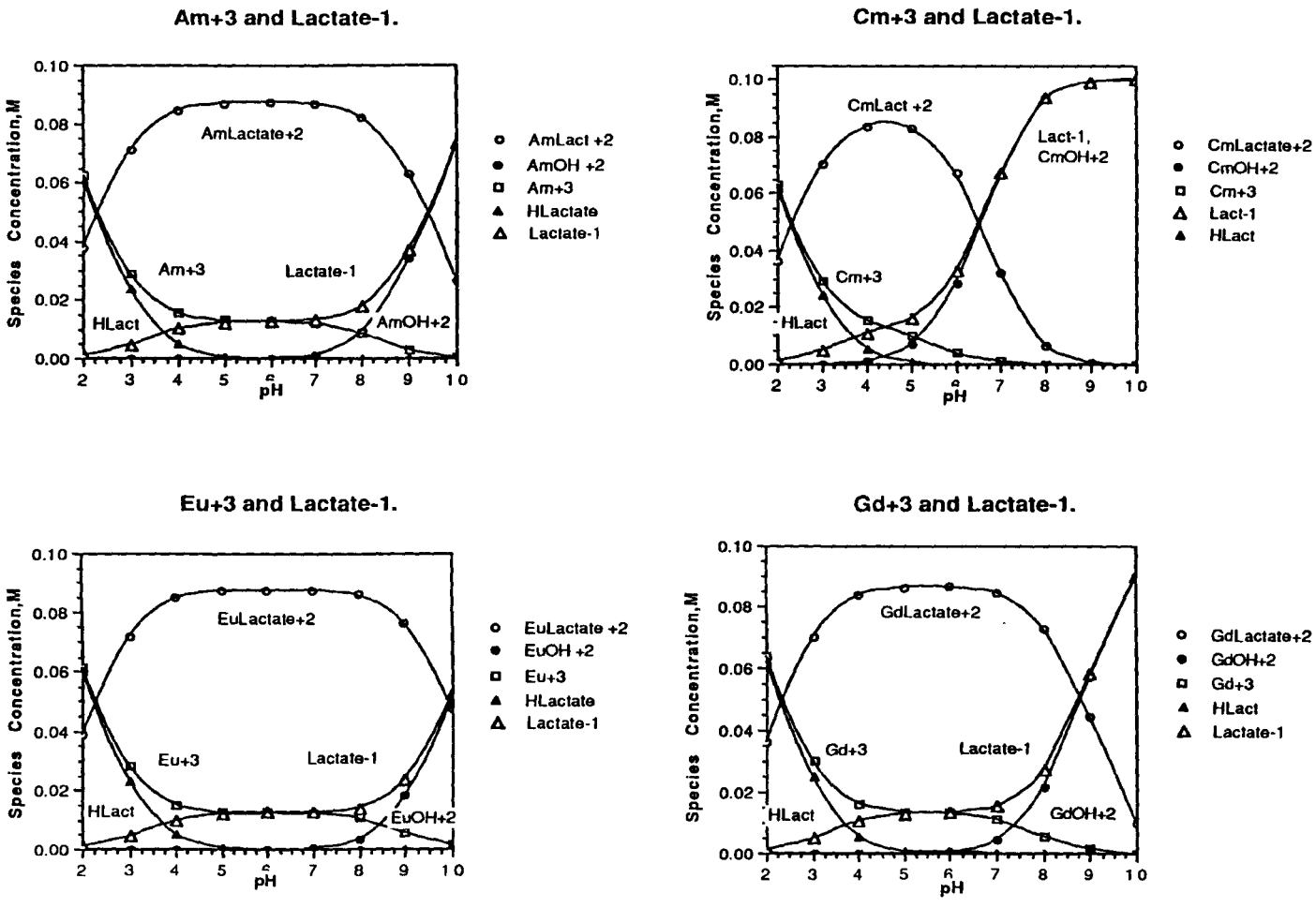


Figure 1. Speciation profiles for Am(III), Cm(III), Eu(III) and Gd(III) with lactate obtained with MINTEQA2 at equal concentrations of metal and ligand of 0.1 Molar.

Publication:

Taylor, D. M. and Gillis, T. M., Attempts to correlate biokinetic behaviour with specific physico-chemical parameters within chemical families: Alkali metals and lanthanides. Radiation Protection Dosimetry, submitted.

Head of Project 4: Dr. J.J.McAughey

II. Objectives for the reporting period:

1. Continue work on the effect of speciation and fasting on fractional gut uptake of barium and neodymium in man, as analogues of radium and the actinides respectively.

2. Assess the metabolism of barium and neodymium in man by administration of stable isotopes by ingestion, and by intravenous injection.

3. Develop analytical methods for the determination of hafnium concentrations and isotope ratios in biological samples using inductively coupled plasma - mass spectrometry (ICP-MS).

4. Conduct an animal study involving injection and ingestion of ^{237}Pu, and ^{175}Hf, both γ-emitting radiotracers. Stable isotopes of ^{176}Hf and ^{143}Nd were administered at the same time. This allows the validation of analytical techniques for hafnium measurement, and yields information on the metabolic behaviour of neodymium and hafnium, relative to plutonium. Information is also be gained on the usefulness of stable isotopes of hafnium as a plutonium analogue in humans.

5. Exchange biological samples for the optimisation of analytical methods and of samples from *in vivo* studies for comparison of results between Frankfurt, Milan and Harwell.

Objectives for the next period:

1. Improve analytical methods for the determination of hafnium concentrations and isotope ratios in biological samples using inductively coupled plasma - mass spectrometry (ICP-MS). This will include measurements of Hf in blood, urine, faeces and diet from normal UK residents.

2. Perform a pilot study of Hf gut transfer factors in 2 volunteers.

3. Exchange biological samples for the optimisation of analytical methods and of samples from in vivo studies for comparison of results between Frankfurt, Milan and Harwell.

III. Progress achieved

Background

Stable isotopes have been used as analogues of radionuclides to measure fractional gut uptake and excretion rates in volunteers. Lanthanides (e.g. Nd) and selected other metals (Sr, Ba) have been assessed as non-toxic analogues for the measurement of actinide uptake in humans. This allows improvement of dosimetric models of gut uptake factors for

radionuclides where these are believed to be low and assessing the metabolic differences between adults and critical groups such as children.

There is evidence from both in-vitro and animal studies that Hf exhibits similar biokinetic properties to Pu (Taylor, M.; Lehmann, F.; Planas-Bohne and Seidel, A., Radiation Res., (1983) **95**, 339-358.) such that its use could complement the data available from other analogues such as Nd. Parallel animal studies have allowed the investigation of radionuclide distribution of Hf and Pu between different body compartments, in particular liver and bone.

Neonates form a critical group in the assessment of ^{90}Sr dosimetry as they have a high rate of Ca uptake and Sr is incorporated with Ca into bone; thus stable isotope administrations offer a route to determining uptake in such a critical group.

Analytical

Work has continued using quadrupole ICPMS to determine isotope ratios for the stable isotopes in urine and faecal samples from volunteers. The work has been extended to assess the capabilities of high resolution ICPMS for the measurement of Ca isotope ratios where isobaric interferences from argon hydrides and carbon dioxide hinder low-level measurement with a quadrupole instrument. Success with this method has allowed its application to the measurement of Ca and Sr uptake and excretion in neonates. Aliquots of these samples have been sent to GSF Frankfurt for analysis by TIMS (see Project 1) in order to compare the performance of the three techniques.

Although the chemistry of Hf differs in a number of ways from that of Pu, it can nonetheless serve as a stable analogue of Pu if its biokinetics and properties under physiological conditions are found to be similar. The chemical differences have meant that preparation of Hf solutions for injection/ingestion and ion exchange separation of Hf for subsequent analysis differ from those used for Pu and Nd. Eichrom TRUspec ion exchange columns were used for the separation and preconcentration of Nd, however this method was found to be unsuitable for use with Hf. An alternative method is being adapted for the Hf study (Greenland, L.P. Anal. Chim. Acta, (1968) **42**, 365-70). Hf in solutions prepared for injection/ingestion in an animal study have been found to be unstable towards formation of a precipitate. Therefore, stabilisation of Hf in solution by citrate is currently being assessed. The results of these stability and analytical pre-concentration studies will be circulated amongst the collaborating groups (see Project 1 - 3).

Calcium and Strontium

Following strontium uptake studies in adults, ^{43}Ca and ^{84}Sr were administered to a single neonate (by ingestion). Preliminary data from this study are presented in Figure 1 and indicate that Ca and Sr excretion to urine, follow a similar pattern. These measurements form a pilot study, the results of which are being used to design the next phase which will involve a larger group of neonates.

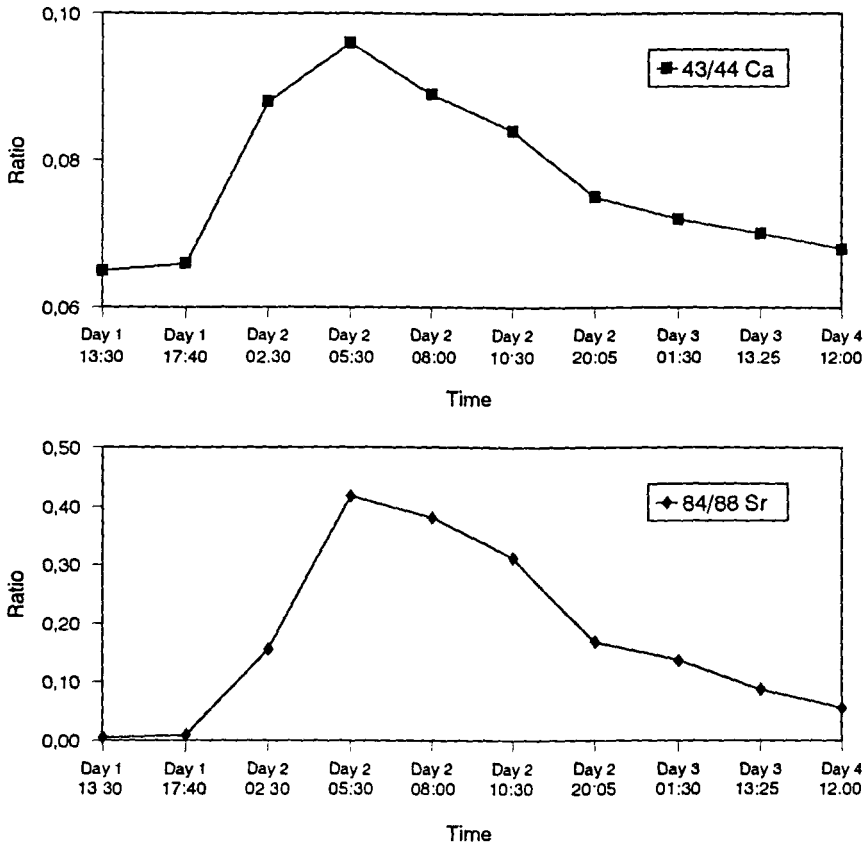


Figure 1: Calcium and Strontium Isotope Ratios in Human Neonate Urine

Barium and Neodymium

Studies of fractional gut absorption have continued in a group of 4 male and 4 female volunteers. Simultaneous oral and intravenous administrations of Ba and Nd (Table 1) have now been given :

a) following an overnight fast
b) following a standard breakfast
c) in the presence of citrate following an overnight fast

Table 1 - Isotope Intakes

Element	Intravenous Intake	Oral Intake
Ba	20 μg ^{134}Ba	200 μg ^{135}Ba
Nd	14 μg ^{145}Nd	150 μg ^{143}Nd

These experiments have been designed to demonstrate whether fractional gut absorption is enhanced following a fast, and whether simple ligands present in the diet, such as citrate, can further enhance uptake. Results are shown in Table 2 for Ba and Table 3 for Nd.

Table 2 - Barium Uptake

Subject (Gender)	f_1 (Fasted)	f_1 (+ Citrate)	f_1 (Fed)	Mean F/U Excretion	Mean Retention
Male					
A	0.20	0.12	0.04	14	-
B	0.43	0.24	-	19	0.11
C	0.29	0.08	0.01	11	0.59
D	0.41	0.32	0.08	10	0.52
Female					
E	0.55	-	0.65	5	0.46
F	-	0.13	-	9	<0
G	0.36	0.17	0.03	21	0.10
H	0.10	0.33	0.13	17	0.12

Table 3 - Neodymium Uptake

Subject (Gender)	f_1 (Fasted)	f_1 (+ Citrate)	f_1 (Fed)	Mean F/U Excretion	Mean Retention
Male					
A	6.3×10^{-3}	1.7×10^{-4}	$< 1 \times 10^{-7}$	0.145	0.93
B	2.1×10^{-4}	5.7×10^{-4}	-	0.138	0.93
C	1.4×10^{-4}	6.5×10^{-4}	9×10^{-5}	0.216	0.92
D	3.0×10^{-3}	4.3×10^{-4}	6×10^{-5}	0.043	0.92
Female					
E	5.3×10^{-4}	-	2.3×10^{-3}	0.096	0.94
F	-	2.3×10^{-4}	-	0.083	0.93
G	3.6×10^{-3}	8.7×10^{-5}	$< 1 \times 10^{-7}$	0.068	0.95
H	4.8×10^{-4}	5.1×10^{-4}	5.8×10^{-4}	0.081	0.92

For Ba, as for earlier Sr data, it has been shown that fractional gut uptake is significantly higher than the adopted ICRP value of 0.1 when subjects have fasted prior to administration. Whole body retention of injected Ba after 7 days showed large variability between individuals. For Nd, fractional gut uptake is also enhanced in individuals who have fasted prior to ingestion, relative to those who had eaten a "standard breakfast" with a number of the fasted uptake values exceeding the adopted ICRP value of 3×10^{-4}. Excretion of Nd was principally via urine with whole body retention found to be 0.94 ± 0.03 after 7 days. Uptake values for Ba and Nd are decreased in the presence of citrate relative to fasting, but not significantly at the 95% confidence level.

Hafnium

^{175}Hf and ^{237}Pu (both γ-emitting isotopes) have been administered orally to rats and the distribution of both Pu and Hf between liver and bone were measured by γ-spectrometry. After 7 days the fraction of ingested Pu in the liver was a factor of 3.7 greater than the fraction of ingested Hf and the fraction of ingested Pu in the femora exceeded the Hf fraction by a factor of 2.0. The parallel study involving injection of the same Hf isotope is now in progress. Stable isotopes of Hf and Nd were simultaneously administered in this study and are currently being measured by ICPMS.

Preparations are now in progress for a study of Hf uptake by volunteers. Background Hf concentrations have been determined in urine and faecal samples from 8 subjects from the Ba/ Nd uptake study. Samples for 3 consecutive days for each subject were analysed for hafnium content. It was noted that isotope ratios were measurable in faecal and urine samples. Hf levels have also been measured in freeze-dried total diet samples and in samples of tap water from a variety of sites in the UK. Results are shown in Table 4 relative to data from 16 Italian adults reported recently (Minoia, C.; Sabbioni, E.; Apostoli, P.; Pietra, R.; Pozzoli, L. Gallorini, M.; Nicolaou, G.; Alessio, L. and Capodaglio, E. Sci. Total Environ., (1990) **95**, 89-105).

These concentration data in humans, plus the distribution data from the animal study are being used to design a pilot study of Hf gut transfer factors in 2 volunteers.

Table 4 - Hafnium Intake & Excretion

Sample	Concentration	Notes
Faeces (n=8 subjects)	400 ng.day^{-1}	Range = <60 - 1000 ng.day^{-1}
Urine (n=8 subjects)	< 45 ng.day^{-1}	n=2, 60, 120 ng.day^{-1}
Diet (n=2)	< 0.5 ng.g^{-1}	
Water (n=12)	< 0.01 ng.ml^{-1}	
Urine (n=16)	490 $\pm$ 220 µg.l^{-1}	Minoia et al, 1990
Blood (n=29)	210 $\pm$ 64 µg.l^{-1}	Minoia et al, 1990

Publication

1. McAughey, J.J.; Vernon, L.; Haines, J.; Sanders, T.; and Clark, R. Fractional gut absorption of strontium, barium and neodymium following administration of stable isotope tracers. Proceedings of the 9th International Conference on 'Heavy Metals in the Environment.' Toronto, 1993.

Progress Report

Contract: **FI3P-CT920060** **Sector: A14**

Title: Radionuclide dosimetry

1)	Noßke	BfS
2)	Kendall	NRPB
3)	Taylor	UKAEA
4)	van Rotterdam	TNO - Delft

I. Summary of Project Global Objectives and Achievements

The objectives of the project are to develop and implement more comprehensive and realistic models for the evaluation of organ and effective doses due to intakes of radionuclides by workers and the general public including doses to the embryo and fetus due to intakes of radionuclides by the mother, and to address the more important uncertainties in the biokinetic and dosimetric models used in radiation protection for evaluating doses to organs from intakes of radionuclides.

In close collaboration with the ICRP Task Group on Age-dependent Doses new age-related biokinetic models for a number of elements have been formulated. In addition this Task Group has proposed a generic model for the biokinetics of lead and the alkaline earth elements. For the determination of the biokinetics of further elements the survey of the literature has continued. Progress has also been made with the formulation of a generic model for the lanthanides.

There is also a literature study concerning the biokinetics of the gastro-intestinal (GI) tract to compare the current ICRP model with possible modifications due to published proposals. In this way compartments necessary to formulate an age-related biokinetic GI tract model will be identified as well as the degree of uncertainty of such models.

Within this project undependently three computational programs are being developed to calculate dose coefficients using very general biokinetic compartment models. Intercomparisons of the results of these codes give a good quality assurance of the codes and of results calculated, for example, for ICRP publications or the CEC basic safety standards.

For the forthcoming ICRP Publication 56, Part 4, work is being performed to develop generic models for doses to the embryo and fetus due to activity intake of the mother. These models are also implemented in the various computational codes.

There is a close collaboration between the groups of this project by work within the ICRP Task Groups of Age-dependent Doses and of Dose Calculations as well as by intercomparison procedures for the results of the various computer codes and by cooperation within the task to calculate effective dose coefficients for the CEC Basic Safety Standards. Within this collaboration there were, for example, exchange visits of Dr. Kendall (NRPB) at BfS as well as of Dr. Noßke (BfS) at NRPB.

Head of project 1: Dr. Noßke

II. Objectives for the reporting period

- Literature study about the gastrointestinal tract to update age-dependent biokinetic data and to implement further compartment into the four-compartment-model of ICRP Publication 30.

- Propositions of new compartments for the gastrointestinal tract model

- Implementation of consideration of daughter nuclides and age-dependence into our computer code DOSAGE

III. Progress achieved including publications

The current ICRP model of gastrointestinal (GI) tract is a four-compartment model including the stomach (ST), small intestine (SI), upper large intestine (ULI), and the lower large intestine (LLI). This model is intended for a reference man which is a 70 kg healthy adult caucasian male and does not allow the variation of conditions as age, gender, the element considered and its physical and chemical form. On the other hand, it has become more important to assess realistic doses to the GI tract because explicit weighting factors have been assigned to the oesophagus, the stomach and the colon.

The aim of our work is to compare this GI tract model with published informations to identify items a new expanded GI tract model should contain. These proposals for the model structure and parameters of a new expanded GI tract model will be compared also to the model being developed by Stubbs.

The physiological and biological conditions in the GI tract are much more complex than it is described by the ICRP model. As a first step it has been investigated which additional compartments a new GI tract model should contain. The mouth and the oesophagus as a part of the ingestion pathway are not included in the ICRP model. Also the absorption of the liquid phase of the elements into the blood and the solid phase into the lymphatic system as well as the excretion pathway through the colon may be described more accurately by additional compartments. Especially the doses to the oesophagus and the colon have become more important by getting own tissue weighting factors in ICRP Publication 60.

As a first result it may be necessary to include the mouth as an own compartment at least for some elements while - because of the fast transit time through it - it seems not to be necessary to include the oesophagus as an own compartment. On the other hand it is planned to estimate the possible underestimation of effective dose by not considering explicitly the biokinetics of the oesophagus. The recommendation of Stubbs of three compartments for the colon appears to be sensible.

Furtheron it will be necessary to consider transfer of activity from the GI tract to the systemic circulation not only from the SI compartment but also from other GI tract

compartments. A question to be considered will be if it is necessary for dosimetric purposes to distinguish between a transfer to blood or to the lymphatic system.

During the previous project we have started to develop a computer code to calculate dose coefficients, excretion rates, and activity contents of various organs and tissues using very general biokinetic models. During the reporting period this code has been expanded to consider daughter products and age-dependence, at the moment only in a simple way leaving constant the biokinetic and dosimetric parameters between the various age groups.

Tests of the code by intercomparison procedures with results of calculations for ICRP Publication 56, Part 2, obtained by Oak Ridge National Laboratory, NRPB and the Ucrainian Scientific Centre for Radiation Medicine showed good agreement of our results with others.

Because of the capability of our code to perform calculations using very general biokinetic compartments model it was possible to perform calculations of the biokinetics of the new ICRP model for the respiratory tract after only minor modifications to our code. Intercomparison of our results with those of the LUDEP code showed very good agreement.

Publications:

None, apart from internal reports about results of intercomparison results.

IV. Objectives for the next reporting period

- Continuation of the identification of important items concerning the model structure and biokinetic parameters for a GI tract model.

- Investigation of appropriate retention functions for the GI tract compartments as well as the possibility to approximate these by exponential functions.

- Assessment of variability of doses due to changes to the GI tract model.

- The implementation of the dosimetric aspects of the new respiratory tract model to our computer code.

- The implementation of the possibility of continuous changing of biokinetic and dosimetric parameters to our computer code as well as the possibility to calculate general recycling models.

Head of project 2: Dr. Kendall

II. Objectives for the reporting period

- The development of computational tools to deal with recent ICRP recommendations on physiologically based biokinetic models. In particular, the respiratory tract model, the recycling bone models for certain elements, and the excretion pathway model.

- The implementation of these models in dose calculations for certain nuclides and the intercomparison of doses with those of other groups.

- Improvements in age-dependent dosimetry, in particular better modelling of growth for some important tissues.

III. Progress achieved including publications

The main efforts have been directed towards work in support of ICRP Committee 2's publications on age-dependent dosimetry. Substantial work has also been performed on the consolidation of software used at NRPB. This has the aim of converging towards a common approach with other research groups, so far as non-essential differences are concerned, while maintaining a diversity of underlying methodology so as to provide quality assurance.

The main area of enhancement of the software has been to allow the more complex physiological models now being developed by ICRP to be incorporated into the NRPB package. A key development has been the EIGEN program. As its name suggests, this uses eigenvalue methods to allow complex recycling models to be solved and the solutions incorporated into the PEDAL2 program. In short, the recycling model is reduced to an ICRP 30-type retention expression for each box of the model. EIGEN and PEDAL2 are thus modules of a suite of programs which can deal with the new generation of complex recycling models. A development path has been planned to allow the package, to deal with the full complexity of age-dependent dosimetry, including time varying biokinetics as well as age-dependent specific effective energies.

Current draft ICRP recommendations (ICRP Publication 56, Part 2) introduce a generic model for excretion pathways. Activity removed from a tissue was previously taken to be simply lost from the body. The new model assigns a fraction of this activity to urinary and faecal excretion, and this leads to additional tissue doses as activity passes through the urinary bladder or lower gut. Although these models are not recycling, they do present a departure from the traditional ICRP 30 models. The EIGEN program is therefore used to simplify the model, in the manner outlined above, prior to dose calculations using PEDAL2.

Calculations of dose coefficients for most radionuclides to be included in Part 2 of ICRP Publication 56 were undertaken. These were compared with calculations by Oak Ridge National Laboratory and by the German Institute for Radiation Hygiene during exchange visits between NRPB and ISH in the summers of 1992 and 1993. This was to provide quality assurance on the dose coefficients. The exercise was very rewarding and suggested ways in

which minor discrepancies in dose coefficients could be avoided, enabling the important discrepancies to be more easily pinpointed.

A new version of the RAPID database has been set up which will be used for future intercomparisons with other research groups contributing to ICRP Publication 56. This differs from the publicly available version of RAPID (NRPB-R245) in a number of ways.

a) ICRP Publication 56 Part 2 biokinetic models are used for all elements except the actinides, alkaline earths and lead.

b) There is now exact agreement with ICRP Publication 56 in total body mass at the six reference ages (3 months, 1, 5, 10, 15 years and adult).

c) Ovaries, testes and breast have been given their own growth functions in addition to the special growth function already in use for thyroid. These masses now also agree with ICRP Publication 56.

d) The photon specific absorbed fractions of Cristy and Eckerman (1987) are used in preference to those of ICRP Publication 23 (1975).

The new database also includes about 60 additional radionuclides. This version of RAPID is not publicly available. However, it represents a significant step forward in terms of harmonisation of approach with other groups.

The NRPB generic model for fetal dosimetry has been further developed. In most cases the available data on biokinetics in the mother and fetus do not support the use of a compartmental model of the kind normally used in internal dosimetry. Thus, a scaling method for estimating fetal tissue doses from the corresponding maternal tissue doses is used. This is based on experimentally observed fetus:mother concentration ratios and other factors such as the time at which distinct fetal tissues appear, average length of gestation, etc. This approach has been accepted in principle by ICRP Committee 2 as the framework for their publication on the subject. More specific models will be developed where the data allow this to be done.

With the recent improvements in speed and storage media on personal computers it has proved possible to put the RAPID database on a PC. This may prove an attractive option to some users, though performance has not yet been optimised. Other groups may find RAPID useful in QA procedures.

Publications:

None, apart from internal NRPB Technical Memoranda, since work for ICRP is not published separately.

IV. Objectives for the next reporting period

- The implementation of the dosimetric aspects of the new respiratory tract model.

- The generation of a full set of CED values for the Euratom Directive on Basic Safety Standards. Extensive QA of NRPB results with those of ISH, and possibly other groups.

- Further refinement of the generic fetal dosimetry model, and development of element specific models in a few cases. This is in support of ICRP Committee 2 work on age-dependent dosimetry.

- Dose calculations for Part 3 of ICRP Publication 56.

Head of project 3: Prof. Dr. Taylor

II. Objectives for the reporting period

- To continue to survey of the post-1970 literature on the biokinetics, and distribution of those elements for which new biokinetic models have not already been published, or prepared for publication, in ICRP Publication 56, Parts 1-3.

- To commence the review and re-evaluation of all the relevant available human and animal data and to prepare new or revised biokinetic models.

- To investigate the possibility and desirability of developing generic models based on chemical similarities for those elements for which adequate biokinetic data are not available.

III. Progress achieved including publications

During the period under review close collaboration has been maintained with the ICRP Task Group on Age-dependent Doses to Members of the Public from Intakes of Radionuclides [AGDOS] and new age-related biokinetic models have been formulated for the elements sulphur, cobalt, nickel, zinc, strontium, molybdenum, technetium, silver, tellurium, barium, lead, polonium and radium; in addition this Task Group has proposed a generic model for the biokinetics of lead and the alkaline earth elements, calcium, strontium, barium and radium. These models have been adopted by ICRP and will shortly be published as ICRP Publication 56, Part 2. New age-related biokinetic models have also been developed for the elements iron, selenium, antimony, thorium and uranium: these models have been submitted to ICRP for adoption and subsequent publication in ICRP Publication 56, Part 3.

The survey of the literature on the biokinetics and biodistribution of elements in animals and humans which have been published since 1970 has continued. The surveys for the elements bismuth, aluminium, gallium, indium, and hafnium have been virtually completed and the first drafts of revised adult biokinetic models for these elements have been prepared. The first draft of a revised biokinetic model for caesium which includes excretion data and which appears to be applicable to the analysis of bioassay data has also been prepared.

Progress has also been made with the formulation of a generic model for the lanthanides. The fifteen elements with Atomic Numbers from 57-Lanthanum to 71-Lutetium form the lanthanide, or rare earth, series. For all the elements in the lanthanide series the M^{3+} state is the most stable, or only known, oxidation state; and their chemical properties exhibit many similarities to the corresponding members of the actinide series. Human biokinetic data for the individual lanthanide elements is fragmentary and the data on biodistribution and kinetics in experimental animals is far from complete. In view of the paucity of human biokinetic information and the chemical similarities between the elements in the actinide and lanthanide series it appeared to be appropriate to consider the formulation of a generic biokinetic model for the lanthanide series, using the information available for individual lanthanide and actinide elements. Since yttrium also shows a close chemical relationship to

the lanthanides and actinides, human data for this element, where available, may also be used in formulating the model.

Information on the gastrointestinal absorption of lanthanide elements in humans is limited to two small studies with ^{147}Pm or with stable neodymium, these suggest absorption (f_1) values ranging from $< 1 \cdot 10^{-5}$ to $\sim 4 \cdot 10^{-3}$. Studies of the absorption of the actinides, thorium, neptunium, plutonium, americium and curium in small numbers of adult human volunteers, yielded f_1 values within the same range. For unknown forms of plutonium, americium and neptunium, ICRP Publication 56, Part 2, recommends a general f_1 value of $5 \cdot 10^{-4}$; the same publication recommends, by analogy with the actinides, an f_1 of $5 \cdot 10^{-4}$ for the lanthanide cerium. Based on these human data and taking account of the strong tendencies exhibited by both lanthanide and actinide ions towards hydrolysis and complex formation in the human gastrointestinal tract, which indicate that free metal ions are unlikely to exist and the elements are most likely to be present almost entirely as unabsorbable hydroxy or oxo-species or as insoluble complexes with food residues; it appeared reasonable to propose $5 \cdot 10^{-4}$ as the f_1 for all chemically unknown forms of the lanthanides, and for yttrium.

Animal data suggest that the deposition of individual lanthanides in the skeleton is directly proportional to their ionic radii, and conversely, liver uptake is inversely proportional to ionic radius. Assuming a similar relationship to apply to humans, and that in the absence of more direct data, the biokinetic parameters for bone and liver developed for the americium model can be applied to the actinides; it was assumed that 80% of the total lanthanide that entered the blood compartment would be deposited in liver and skeleton and, thus, that the following rounded liver:skeleton deposition ratios could be applied to the lanthanides.

Element	Liver:skeleton deposition
Lanthanum	6.0:2.0
Cerium, Praseodymium, Neodymium, Promethium	4.5:3.5
Europium, Gadolinium, Terbium	3.5:4.5
Dysprosium, Holmium, Erbium	2.5:5.5
Thulium, Ytterbium, Lutetium, Yttrium	1.5:6.5

Based on autoradiographic evidence for ^{90}Y in rabbit bone, it is proposed that half the skeletal deposition be assigned to cortical bone surfaces and half to trabecular surfaces.

This model is still being completed and refined, but in the absence of more detailed information for the lanthanides on uptake in the gonads and other tissues, and on the rates of translocation from bone and liver and on excretion, the biokinetic parameters developed for americium have been applied to the proposed generic lanthanide model.

The revised biokinetic models which have been developed for bismuth, aluminium, gallium, indium and hafnium, together with the generic model for the lanthanides will be submitted to ICRP Committee 2 for discussion and approval.

Publication:

Taylor, D. M. and Gillis, T. M. Attempts to correlate biokinetic behaviour with specific physico-chemical parameters within chemical families: Alkali metals and lanthanides. *Radiation Protection Dosimetry*, submitted.

IV. Objectives for the next reporting period

- To continue the reviews of the post-1970 literature on the biokinetics and biodistribution of those elements not already reviewed.

- To prepare the updated biokinetic models required for the revision of ICRP Publication 30, including further development of a generic model for the lanthanides.

- To investigate the feasibility of expanding the ICRP biokinetic models to include excretion data and to make them suitable for calculating body contents from excretion data provided by bioassay.

Head of project 4: Dr. van Rotterdam

II. Objectives for the reporting period

The objectives for the reporting period were to develop general models for the biokinetic behaviour of inhaled, ingested or injected radionuclides. These compartmental models should be suited to analyse large systems with residence times of widely different magnitude, in order to properly study the influence of newly obtained experimental data on the behaviour of respiratory tract tissues and to evaluate, among others, the effect of different gastrointestinal properties on the cumulated activity.

III. Progress achieved including publications

Based on the assumption that the processes underlying the deposition and clearance of radionuclides in the organs can only be specified in discrete time steps, we reformulated the biokinetic processes by means of difference equations. We, hence, refrained from the use of redundant differential equations which, anyhow, have to be replaced by discrete approximations because only the latter can be manipulated numerically.
A straight-forward algorithm for the solution of coupled difference equations has been developed and has been incorporated in a computer system COMPART. This system enables the evaluation of outputs of linear multi-compartmental models of arbitrary complexity (including recycling) which can be excited instantaneously or by means of time-varying forcing functions. The program is menu directed and can be operated in an interactive way. It runs on any personal computer with an MS-DOS operating system and a hard disk with a capacity of, at least, 20 MB. Input, output and system information is stored in files and can be easily manipulated by means of simple spreadsheet techniques. Input, output and computation sampling intervals can be chosen as well as the time span of the output signals. Simple plotting routines enable the representation of the different signals as a function of time.

With COMPART a number of simulations of well known metabolic processes have been performed. The time behaviour of the activities and cumulated activities could be accurately estimated. Hence, it can be concluded that COMPART can be a valuable tool for the computation of retention and clearance of radioactive compounds.

Until to date, the question of model uncertainty has not been answered. One aspect of this problem concerns the physiological relevance of the model parameters and the answer can only be obtained by further physiological experimentation. Another aspect, however, concerns the intrinsic stochastic behaviour of the radioactive compounds in the body. We therefore developed a stochastic algorithm and implemented this in COMPART as a second computational mode. The algorithm is based on the assumption that residence times of elementary amounts of a radioactivity in an organ or part of an organ, have no fixed value but are governed by a stochastic process. In the first order description of biokinetics the distribution of the residence times will be negative exponential. A second probability, determined by the coupling between the compartments, describes the transport of the activity from one to another compartment. In the stochastic mode of COMPART it is therefore possible to simulate different realisations of the same biokinetic process. These simulations

allow the user to judge whether measured activities have an accidental character or are caused by significant kinetic properties of the organs under study.

Finally it has to be mentioned that these stochastic simulations can be performed with integer numbers so that the computation does not introduce any additional inaccuracies.

Publication:

van Rotterdam, A. An algorithm for the evaluation of large compartmental models with residence times of widely different magnitude. To be published.

van Rotterdam, A. A stochastic simulation technique to model the probabilistic behaviour of radioactive compounds in the body after ingestion or inhalation. To be published.

IV. Objectives for the next reporting period

In the next year the newly developed compartmental organ models will be implemented in interactive computer codes for dose evaluations. With such programs it will be possible to simulate, as accurately as possible, deposition, clearance and dosimetry in the different organs for which new physiological information has become available. Collaboration with the other participants of the contract, especially in the field of biological experimentation, will be of major importance in this respect. Furthermore it is of importance to compare the simulation results to be obtained with those already existing and with data obtained by means of other computer programs, such as e.g. LUDEP.

Progress Report

Contract: **F13P-CT920064a** **Section: A14**

Title: Inhalation and ingestion of radionuclides.

1)	Bailey	NRPB
2)	Stahlhofen	GSF
3)	Roy	CEA - FAR
4)	Patrick	MRC
5)	Stradling	NRPB
6)	Iranzo	CIEMAT

I. Summary of Project Global Objectives and Achievements

Introduction

This project is an extension and development of part of the previous co-ordinated project "Inhalation and Ingestion of Radionuclides" (Bi6-0347b) carried out within the CEC/NRPB Association Agreement, by the first four of the organisations listed above. The new project focuses more closely on the inhalation route of intake. Since it started in July 1992 two more participants have joined it. Drs. Iranzo (CIEMAT) and Stradling (NRPB) had submitted a separate joint proposal to investigate the biokinetics of actinides associated with soil contaminated by the nuclear weapons accident at Palomares, Spain, in 1966. Their involvement extended the scope of the project to the application of models to particular situations, including the determination of relevant site-specific parameters. As described below, it is likely that at least four more participants will join during the next period.

Objectives

The objectives of the project are to address the more important uncertainties in the models used in radiation protection for evaluating doses to internal organs from intakes of radionuclides, by inhalation and ingestion; to develop and implement more comprehensive and realistic models for relating exposures and intakes to organ doses and monitoring results for workers, and for calculating the distribution of doses among the general population. The emphasis is on inhalation, and for this route the project includes experimental studies aimed at providing basic data required to improve models.

An important part of the work is linked to the development of ICRP models and recommendations relating to the dosimetry of internally deposited radionuclides. This is a particularly active area at present and will continue to be so for the next several years, with the revision of the respiratory tract and GI tract models, reference man, and age-dependent biokinetic models for individual elements. These are expected to lead to further parts of ICRP Publication 56, in particular application of the new respiratory tract model to calculation of dose coefficients for members of the public, and to complete revisions of ICRP Publications 30 and 54.

Achievements

During this reporting period progress has continued much as anticipated in all areas. Considerable effort went towards the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection, of which three participants (Bailey, Roy, Stahlhofen) were members. During this period further revisions were made to the model, its supporting documents and software, to take account of reviews by ICRP Committee 2 and the Main Commission, and to incorporate new information. With adoption of the model by ICRP in April 1993, emphasis has shifted to implementing it: development and extension of software, and selection of parameter values for various situations. The model was applied to predict the effective dose per unit exposure to radon decay products, and the result compared with that inferred from epidemiological studies of miners. An analysis of the sensitivity of this dose calculation to uncertainties in the model parameters was conducted.

Experimental work within this project continues to address areas of uncertainty highlighted by development of the new model. The bronchial epithelium is thought to be of particularly high radio-sensitivity, and investigations of particle retention in this region have continued. Further studies in which subjects inhaled radiolabelled particles as a small bolus at the end of a breath were conducted at GSF. Recent results, using particles of various sizes and densities, indicate that the fraction of deposited particles that is not cleared rapidly by mucociliary action decreases with increasing particle geometric size. Complementary studies continued at MRC to investigate the slow clearance mechanisms. Measurements on the tracheas of F-344 rats, which indicated that approximately 60% of the surface is non-ciliated, were extended to another rat strain (HMT), to mice (CBA), and guinea pigs. The work was extended to measurements on longitudinal as well as transverse sections, and to analysis of the distribution of lengths of non-ciliated areas. Further study was conducted of the clearance of gold particles deposited in the lungs by alveolar microinjection, reported previously. The biokinetics of intravenously injected gold in the rat were determined and used to assess the contributions of dissolution and particle transport to alveolar clearance.

Uncertainty and, indeed controversy, over the extent to which the bronchial tree is cleared rapidly by mucociliary action has also drawn attention to the limited ability of current mechanistic models of particle deposition to predict the regional deposition of particles inhaled as a bolus. Model experiments at GSF are planned to investigate directly the behaviour of an inhaled bolus in the respiratory tract.

Human experimental studies have also continued to measure:

- Physiological parameters and the partition between nose and mouth breathing in healthy adults during graded exercise, following construction of the apparatus required.
- Oral and nasal peak flow-rates in children (male and female).
- Respiratory tract deposition of monodisperse (1, 2 and 3 μm) particles inhaled by children, healthy adults (male and female), and adults with impaired lung function. The aerosols were inhaled under the same, controlled, conditions to investigate the effect of airway dimensions.
- Respiratory tract deposition of ultrafine particles (30 nm) inhaled by healthy adults.
- Aerosol bolus dispersion and pulmonary function in smokers and non-smokers.

- Alveolar retention and macrophage activity using magnetopneumography.

The first phase of a collaborative study between CIEMAT and NRPB on the radiological implications of inhaling ^{239}Pu and ^{241}Am present in soil contaminated by the nuclear weapons accident at Palomares, Spain in 1966, was completed. The chemical, radiochemical and mineralogical characteristics of samples of contaminated soil have been determined, *in vitro* dissolution studies were performed, and samples selected for *in vivo* biokinetic studies. An initial study was conducted of the biokinetics of ^{239}Pu and ^{241}Am present in soil fractions after deposition in rat lung, and the implications for individual monitoring of persons exposed to such dusts assessed.

Meetings

Drs. Bailey (NRPB), Patrick (MRC), Roy (CEA), Scheuch (GSF) and Stradling (NRPB) met during the EULEP General Assembly, Reisensburg, FRG, March 1993. Drs. Scheuch and Koblinger (KFKI) met at the Congress of the International Society for Aerosols in Medicine, Garmisch-Partenkirchen, April 1993. Dr Stradling visited Drs. Espinosa and Iranzo at CIEMAT in Madrid in July 1993, to finalise a joint report on work carried out on this project, and agree the programme for the extension to 1995. Formal contractors' meetings are planned for Bath UK, 13 September 1993, and Neuherberg, FRG, 28/29 March 1994. The former is to be a joint meeting with the Project "Radionuclide dosimetry" co-ordinated by Dr. D Nosske; the latter with both that project and "Assessment of internal dose from plutonium and other radionuclides using stable isotope tracer techniques in man", co-ordinated by Dr. P Roth. The meeting in September 1993 at Bath immediately precedes the Workshop on Intakes of Radionuclides; Detection, Assessment and Limitation of Occupational Exposure, which is sponsored by the CEC and at which several papers based on work supported by this contract will be presented.

Extension

Negotiations are at an advanced stage for four further participants to join this project. Two were included in the original submission in 1992: PL920091-A14 No8 (Popplewell, NRPB, UK) and PL920091-A14 No9 (Strong, AEA, UK). Popplewell is conducting human volunteer studies of the gastro-intestinal uptake and biokinetics of radionuclides. The current study is of the absorption and long term urinary excretion of plutonium, using the long-lived isotope ^{244}Pu. Strong will carry out measurements of the deposition of hygroscopic particles in the human respiratory tract. Deposition of monodisperse particles in women will be determined, and compared with results previously obtained in men. The third was a separate submission, PL920181-A14 (Johnston, Institute of Occupational Medicine, IOM, UK), entitled "Development and testing of dust sampling instruments commensurate with application of the new ICRP lung dosimetry model in Europe." The emphasis is on development of a high flow-rate personal air sampler. The fourth, Dr. Koblinger (KFKI, Budapest, Hungary) concerns the development of mechanistic models of aerosol particle deposition in the respiratory tract. This complements the existing programme which mainly involves experimental studies and phenomenological modelling. It will bring state-of-the-art deposition modelling into the project.

Following a CEC initiative (PECO) to promote participation is existing programmes by research organisations in Central and Eastern Europe, several applications (from Bulgaria, Poland and Roumania) were submitted to join this project.

Head of project 1: Dr. Bailey

II. Objectives for the reporting period

i. Refinement of the models for respiratory tract deposition, clearance and dosimetry of inhaled radionuclides for the new ICRP respiratory tract model, with particular consideration of aspects of slow clearance and airway wall retention in the bronchi and bronchioles.

ii. Specification of default parameters for the new model, with particular consideration of parameters representing the time dependent absorption of material to blood.

iii. Investigation of the predictions of the model for doses received as a result of exposure to radon, and comparison with doses inferred from the results of epidemiological studies.

iv. Development and quality assurance of software to implement the new model.

v. Continuation of preparations for experimental studies of the kinetics of clearance of inhaled particles from the human nasal passage.

Objectives for the next reporting period

i. Specification of default absorption parameters for a range of the more important elements and compounds in radiological protection, and specification of default particle sizes for both occupational and environmental exposure.

ii. Further development of the software implementing the new model, with emphasis on handling radioactive decay chains, incorporation of age dependence and inhalation of gases and vapours, and development for bioassay interpretation.

iii. Commencement of human volunteer studies of the effects of particle size and breathing pattern on particle clearance from the human nasal passage using ^{99m}Tc-labelled aerosols.

III. Progress achieved including publications

Collaboration in the work of the ICRP Task Group on Human Respiratory Tract Models for Radiological Protection[1,2] continued. The main changes to the model made during this reporting period were in the treatment of deposition and clearance. These were :

- deposition in each region of the respiratory tract is now treated explicitly as a series of filters in which deposition occurs during both inhalation and exhalation.

- the slow clearance of particles from the bronchial and bronchiolar regions was made size dependent, as indicated by the recent studies at GSF Frankfurt.

- the fraction of material deposited in the alveolar region subject to very long-term retention was decreased from 33% to 10%.

Contributions were made to the completion of the ICRP Task Group report, which was approved by the ICRP Main Commission in April 1993; it will be published as ICRP Publication 66[3].

Following acceptance of the model by ICRP, the emphasis of modelling work has now shifted to its application. Parameters which determine the rate of absorption of material from the respiratory tract to the systemic circulation for each element (and some compounds) are to be selected, to enable dose coefficients to be calculated both for workers and members of the public. In preparation for a full review of absorption parameters, a pilot study using caesium as an example has been conducted. The literature on caesium inhalation has been reviewed and absorption parameters for use with the new model calculated for several chemical forms.

A review is also being carried out of particle sizes typical of both occupational and environmental exposures to inhaled radionuclides. It has often been suggested that the default particle size for occupational exposure should be greater than the 1 μm currently assumed by ICRP. Initial results of the review of published measurements of size distributions of radioactive aerosols in workplaces imply that a representative value would be closer to 5 μm.

Inhalation of radon decay products is the dominant source of radiation exposure at home and at work, and the predictions of the new model for doses from this exposure source are therefore of great interest. Predictions for dose per unit exposure, expressed in terms of sieverts per Working Level Month (Sv/WLM), have been compared with dose per unit exposure inferred from the results of epidemiological studies of miners. This comparison is assisting in making judgments regarding the validity of the model for radon dosimetry[4].

The personal computer (PC) program LUDEP (LUng Dose Evaluation Program) developed to implement the new respiratory tract model has been adapted to implement all the changes made to the model. A major enhancement was the addition of a database to allow the radionuclide decay data from ICRP Publication 38 to be used for dose calculations. In addition, the deposition module was restructured to enable gases and vapours to be incorporated in future. Work is progressing on the development of mathematical methods for biokinetic modelling of radioactive progeny where it can be assumed that the progeny have the same biokinetic behaviour as the parent[5]. LUDEP was used in the assessment of the implications of the final version of the new model, which was included in the Task Group report.

Work has been completed on production, documentation and quality assurance of the first commercial version of LUDEP[6,7], which is due to be released in November 1993. It is appropriate for adults, and for single radionuclides or radionuclides with simple decay chains. The user can calculate doses or dose-rates to each region of the respiratory tract at any time after intake. All of the biokinetic models from ICRP Publication 30 have been incorporated, allowing doses to other organs to be calculated. Parameter values recommended by ICRP are installed as defaults, but the user can change many of them for specific situations.

The development of techniques for the generation and labelling of monodisperse aerosols has continued. Monodisperse polystyrene aerosols have been produced using the spinning top aerosol generator (STAG) in the size range 1 - 8 μm, with efficiencies of over 25% for particles up to 5 μm. The vibrating orifice aerosol generator has been used to create monodisperse aerosols from 2 - 11 μm with satisfactory efficiency. Aerosols produced using the STAG have been successfully labelled with ^{99m}Tc. The commissioning of the laser photometer has begun. Engineering control measures have been implemented to ensure the protection of the operator and the subject from any optical hazard.

Work has continued on upgrading the low background *in vivo* measurement facilities. Although development of *in vivo* measurement facilities is not carried out under this contract, they will be used for the inhalation studies. The commissioning of an array of high resolution germanium detectors and the replacement of the data acquisition system have now been completed, and a programme of calibrations has commenced. Specifically, calibration work is well-advanced for the measurement of inhaled low-energy photon emitters in the lung[8].

Publications

1. Bailey, M.R. *New ICRP Human Respiratory Tract Model.* Radiol. Prot. Bull. **144** 22-29 (1993).

2. Bailey, M.R. *The new ICRP model for the respiratory tract.* Submitted to Radiation Protection Dosimetry, Proceedings of the Workshop on Intakes of Radionuclides : Detection, Assessment and Limitation of Occupational Exposure (Bath, UK. September, 1993).

3. International Commission on Radiological Protection. *Human Respiratory Tract Model for Radiological Protection.* ICRP Publication 66. (Oxford: Pergamon Press) (1994, in press).

4. Birchall, A. and James, A.C. *Uncertainty analysis of the effective dose per unit exposure from radon progeny using the new ICRP lung model.* Submitted to Radiation Protection Dosimetry, Proceedings of the Workshop on Intakes of Radionuclides : Detection, Assessment and Limitation of Occupational Exposure (Bath, UK. September, 1993).

5. Birchall, A. and James, A.C. *A rapid method for handling radioactive progeny and its application to modelling thorium in the lungs.* Submitted to Health Physics.

6. Jarvis, N.S. and Birchall, A. *LUDEP 1.0, a personal computer program to implement the new ICRP respiratory tract model.* Submitted to Radiation Protection Dosimetry, Proceedings of the Workshop on Intakes of Radionuclides : Detection, Assessment and Limitation of Occupational Exposure (Bath, UK. September, 1993).

7. Jarvis, N.S., Birchall, A., James, A.C., Bailey, M.R. and Dorrian, M-D. *LUDEP 1.0. Personal computer program for calculating dose to the respiratory tract and to other organs of the body using the new respiratory tract model of the ICRP.* Chilton, NRPB-SR264 (1993) (London, HMSO), submitted for publication.

8. Smith, J R H., Marsh, J.W., Etherington, G., Shutt, A.L. and Youngman, M.J. *Evaluation of a high purity germanium detector body monitor.* Submitted to Radiation Protection Dosimetry, Proceedings of the Workshop on Intakes of Radionuclides : Detection, Assessment and Limitation of Occupational Exposure (Bath, UK. September, 1993).

Head of project 2: Dr. Stahlhofen.

II. Objectives for the reporting period.

i. Aerosol bolus dispersion and retention in casts, lung models and diseased lungs.

ii. Mucociliary clearance measurements in humans after bolus inhalation.

iii. Magnetometric measurements of Fe_3O_4 particles *in vivo* and *in vitro* (retention, magnetic relaxation, intracellular viscosity, macrophage mobility and particle uptake).

iv. Production, measurements and inhalation studies with ultrafine particles.

Objectives for the next reporting period

i. Aerosol Bolus Measurements. Patients with lung disease will be investigated with the bolus inhalation technique, to study ventilation disturbances in the human lungs. Measurements and calculations will be performed to study the dispersion mechanisms in the oral cavity and larynx.

ii. Magnetopneumographic measurements will be performed on patients with selected lung disease, e.g.: idiopathic fibrosis, pneumoconiosis, scleroderma, systemic lupus erythematodes. With these measurements new insight will be obtained into abnormal and disturbed mechanisms of the defense system of the human lung (where pulmonary macrophages are involved).

iii. Mucociliary Clearance Measurements. Mucociliary clearance after aerosol bolus inhalations with different materials will be measured. Mucociliary clearance will be investigated in healthy normal subjects to find the range of normal clearance efficiency and the measurements will be extended to patients with disturbed lung function.

iv. Ultrafine aerosols. Shallow bolus inhalations with ultrafine particles will be performed, and the clearance of these particles from the airways investigated.

v. Charged and Hygroscopic Aerosols. Charged and hygroscopic aerosols will be produced and inhalation studies with these particles conducted, measuring deposition sites and clearance behaviour.

III. Progress achieved including publications

Aerosol Bolus Dispersion and Retention

The aerosol bolus dispersion and retention of particles in the human airways were measured, varying particle size (0.8 - 6 µm) and penetration of the boluses into the airways. The movement of the heart increased the dispersion of inhaled aerosol particles during breathhold for particles smaller than 2 µm. Larger particles were deposited more efficiently in the airways by sedimentation during breathholding periods. Comparing the experimental results from recovery measurements with theoretical model calculations, it

could clearly be shown that only a small fraction (< 2-3%) of shallow inhaled boluses could have reached the alveolar space during inhalation. Thus the bolus technique enables us to inhale aerosol to the tracheobronchial region, exclusively.

Mucociliary Clearance Measurements

After aerosol bolus inhalations the clearance of inhaled particles was measured by following the decay of a radioactive label. Particles of different materials and sizes were inhaled to shallow lung depths, so that they exclusively reached the conducting airways. It was found that the effectiveness of mucociliary clearance is particle size dependent. Large particles (> 5 µm geometrical diameter) were cleared more effectively than smaller particles. In Fig. 1 the dependence of the slowly cleared fraction (A) as a function of the geometric particle diameter is shown.

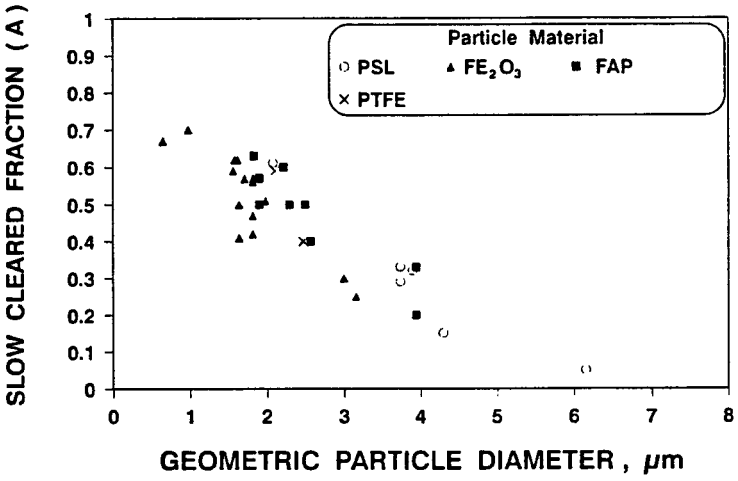


Fig. 1 : Slow cleared fraction (A) of inhaled shallow boluses as a function of the geometric particle diameter. The inhaled aerosol materials were : PSL : Polystyrene, Fe_2O_3 : iron oxide, FAP : Fused aluminosilicate, PTFE : Teflon. V_F : volumetric front depth of the inhaled boluses.

Ultrafine Particles

Aerosols of ultrafine, monodisperse, inert ^{111}In oxide particles were inhaled during steady state breathing (particle size about 30 nm). The chosen tidal volume was 1 L, flow rate : 250 cm^3/s. Only about 4-7% of the inhaled particles were cleared within the first few hours. More than 90% were cleared slowly.

In a following study the ultrafine particles were inhaled as a bolus to a very shallow lung depth (V_F = 42 cm^3). In Fig. 2 it can be seen that only about 20% of the aerosol was cleared within the first day, the other particles remained longer in the airways. Total deposition of this aerosol bolus was 7%.

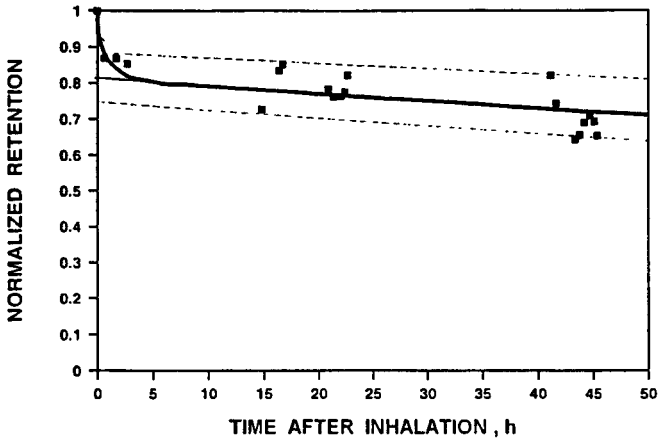


Fig. 2: Lung retention of aerosol particles (d = 30 nm) after bolus inhalation to V_F = 42 cm^3.

Magnetometric Measurements

Magnetic microparticles were used in the human lungs to investigate long term particle retention and particle relaxation, alveolar macrophage mobility and intracellular viscoelasticity. Parallel studies of these dynamic parameters were made with a macrophage like cell-line J774. These *in vitro* investigations showed similar behaviour to that observed *in vivo*. This allowed us to conclude that the magnetic particles within the lungs monitor macrophage behaviour. Additionally, the *in vitro* studies allowed us to verify a model that described relaxation as a rotational diffusion process. This provided a parameter for macrophage mobility in the form of a cellular energy, that was three orders of magnitude higher than thermal energy.

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Head of project 3: Dr. Roy.

II. Objectives for the reporting period.

Evaluation of exposures and intakes of inhaled radionuclides in workers and in members of the general population requires the use of their ventilation rates and breathing patterns. For modelling purposes standard values have been calculated from experimental published data. In order to validate the models and to improve their reliability, we proposed to measure directly in groups of volunteers some of these parameters and their individual ranges of variability.

1. Measurement of ventilation and breathing mode in healthy adults and children. These are especially important with regard to the total and relative fractions of inhaled particles deposited in extra-thoracic and thoracic airways. Deposition is linked to the distribution of ventilation that is generally nasal at rest, and becomes oro-nasal when ventilation needs increase during exercise.

2. Measurement of respiratory tract deposition of inhaled inert particles in Caucasian subjects: healthy adults and children, as well as restrictive and obstructive patients. Search for relationships between individual deposition data, and respiratory function volumes and airflows.

Objectives for the next reporting period.

We propose to continue human experimental studies to ascertain central values and to evaluate variability in physiological parameters.

1. Partitioning of airflow between nose and mouth during incrementally graded exercise will be measured in more adults, and in children younger than 15 years old. The results will be related to airflow / volume curves observed through nose and through mouth successively during forced vital capacity manoeuvre, and to nasal and oral resistances to airflows.

2. Experimental measurements of breathing rates.

In order to validate standard values of daily air volumes inhaled by workers and by members of the public, ventilation rates will be measured in volunteers wearing devices that will record thoracic respiratory movements while the volunteers perform their usual activities.

3. Deposition of inhaled inert particles will be measured in the airways of subjects from various ethnic groups and compared with the data previously obtained in Caucasians with the same methodology.

III. Progress achieved including publications

1. <u>Ventilation parameters and breathing modes.</u>

Among the most useful parameters to include in predictive deposition models are the ventilation rates at rest and at various levels of exercise, and the patterns by which

airflows are inhaled through extra-thoracic airways, nose and mouth, and their dependence upon age and exertion.

1. 1. Measurement of oral and nasal peakflows in children.

A major component of airflow partitioning between oral and nasal routes is the maximal permeability of each one to respiratory airflows.

In 45 children, aged 7 to 17 years, 24 males and 21 females, oral and nasal peakflows were measured during forced vital capacity manoeuvres. Data were plotted against age and body height and fitted to linear regression functions. Both genders' data were equally well explained by body size and age and gave very similar adjustments. They were therefore finally combined to give the following equations:

Oral peakflow :

PF (or) = 0.083 H (cm) - 0.021A (y) - 7.43 (r = 0.78)

This regression is close to the data published by other authors, (Knudson RJ, Slatin RC, Lebowitz MD, Burrows B. Maximal expiratory flow / volume curve. Normal standards, variability, and effects of age. *Am Rev Respir Dis,*. 1976, 113, 587-600).

Conversely, the following relationships do not seem to exist in the literature:

Nasal peakflow :

PF (na) = 0.0049 H (cm) - 0.0004 A (y) - 0.196 (r = 0.49).

Like the oral ones, nasal peakflows are explained by height and age but a lower correlation coefficient is observed. This is probably an independent effect of nose features.

Correlation between data of nasal and oral peakflows : (Figure 1).

PF (or) = 0.051 PF (na) + 0.28 (r = 0.53)

1. 2. Measurements of the distribution of ventilation through nose and mouth in healthy adults performing graded exercise.

A group of ten adult volunteers, of both genders, performed incrementally graded exercise in an appropriate device, set up for this purpose. Two Fleisch pneumotachographs in masks, recorded separately the oral and nasal flowrates of the subject, first at rest, then while exercising on an ergometer bicycle. The subject was requested to increase his/her effort of **25 watts** every one or two minutes. **Figure 2** describes the nasal and oral partitioning of tidal volume during the test in subject n°1. Cardiac frequency, diastolic and systolic blood pressures were simultaneously recorded. Total nasal resistances and flow / volume curves during maximal vital capacity manoeuvres by oral and by nasal breathing were measured in every subject.

Data obtained with these subjects are summarised in **Table 1.** In all of them, oral breathing supplemented airflow through the nose, at levels of exercise which varied betwen individuals, but was well defined and reproducible in the same subject. Mouth-breathed airflow fractions were dependent upon the total ventilation required. At the maximal work load completed by our subjects, they ranged from **30 to 67 %.** In six of them, breathing

abruptly became oro-nasal at ventilation levels from **38 to 65 L. min^{-1}** (switching point). In the four others, implementation of airflow by oral breathing was already important at rest, and increased regularly towards maximal ventilation. In these subjects, the oral to nasal airflow ratios, measured by the flow / volume, quick and simple method, were higher than in the six others. This could indicate an impaired respiration airflow by the nose, and explain the need for oronasal ventilation even at low levels of exertion. However, any relationship with high local values of nasal resistance to airflow was not evident in these subjects.

2. Experimental inhaled particle deposition in the airways of healthy adults and children and in adults with impaired lung function.

Total deposition fractions of an aerosol inhaled through the mouth and containing three sizes of monodisperse particles (**1.2, 2.3, and 3.3 µm** aerodynamic diameter) have been previously measured with controlled respiratory parameters. All the subjects were required to perform the test with the same tidal volume, **VT = 0.5 litre,** and inspiratory times, **t_i = 1.5 seconds**, because meaningful observations of variations in aerosol deposition among subjects with various lung dimensions were possible only if the breathing pattern was kept constant for all of them. This was asked of four groups of volunteers with the aim of studying the influence of lung volume and flowrate, and therefore of age and body size, and eventually of pathology. The required parameters, VT and t_i, were correctly completed by almost all of them, and their deviations were smaller than **10%**; the highest were observed in obstructive subjects.

The differences in deposition data obtained in **11 men and 7 women, out of 18** healthy adult non smokers were not found to be significant by the Wilcoxon non parametric test between the two genders, possibly because the numbers of subjects were too small. Men and women had similar values of Functional Residual Capacity, **FRC**, but women who had smaller values of Forced Expiratory Volume in one second, **FEV_1**, also had higher deposition of **3.3 µm** particles (**47%** versus **44.1%**). These differences being non-significant, data of both genders were treated as a whole in all groups, **13 children** aged **8 to 15 years, 15 restrictive and 15 obstructive** adult patients, without separating them into sub-groups by gender.

Mean deposition values of the four studied groups are summarised in **Table 2.** All the deposition data are lower in healthy adults, but the differences are not always significant, especially for obstructive patients, whose data are the most dispersed. This is probably related to their inability to keep their breathing pattern completely consistent with the required parameters, but also, this could be a consequence of their high tobacco consumption, which is absent in the other groups.

Inverse linear correlations were found between all deposition data and lung function.

In healthy adults, children and restrictive patients, Total Lung Capacity, **TLC, FRC** and **FEV_1** presented significant correlation coefficients with deposition, especially for **2.3 and 3.3 µm** particles, (**Figure 3**) that was linearly increased in lungs with reduced volumes.

In obstructive patients, inverse correlations of deposition of the three particle sizes

were only observed with FEV_1. Multiple correlations with FEV_1 and FRC or TLC did not improve the significance of the correlation coefficient ; this shows the predominant effect of airway obstruction over lung dimensions in these subjects.

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Fig. 1 : Oral / nasal peakflow in children

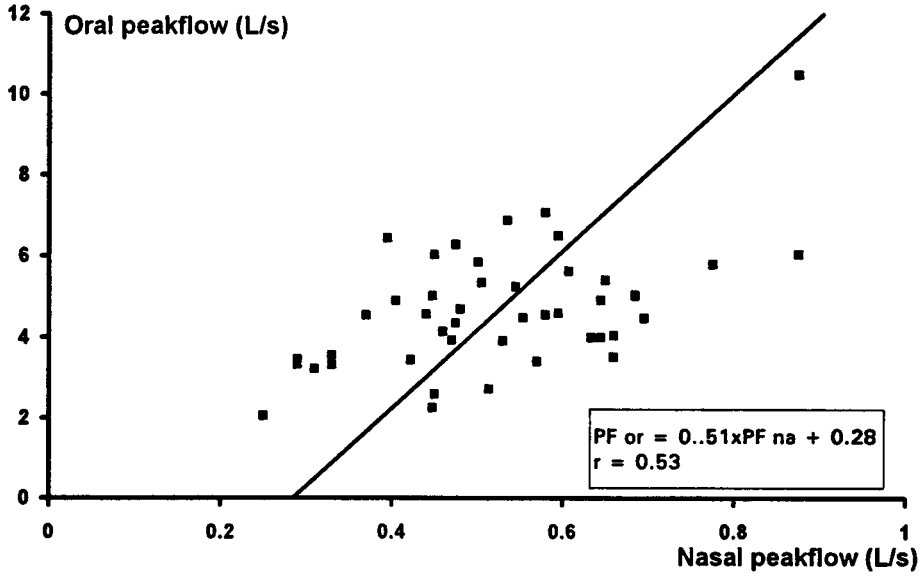


Fig. 2 : Oro / nasal airflow partitioning during increasing exercise

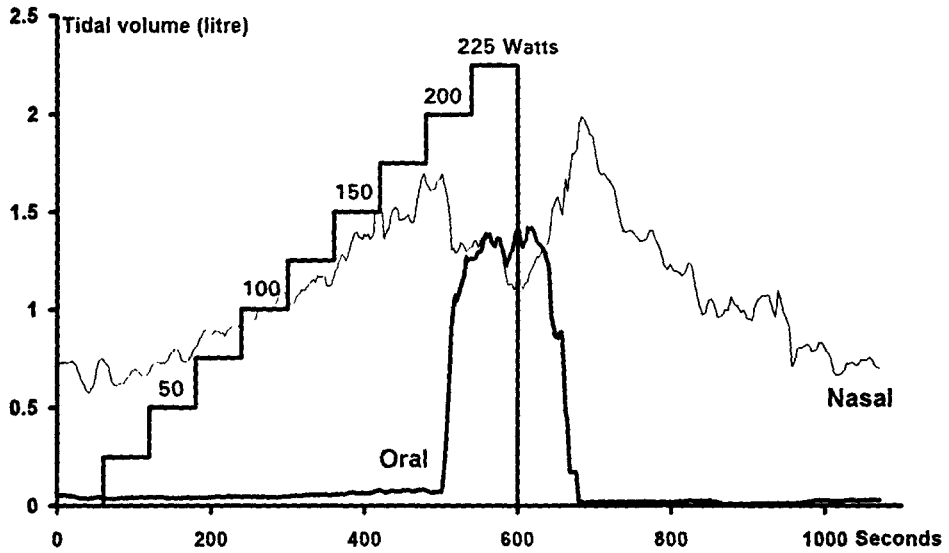


Fig. 3 : Regression of experimental deposition as a function of FRC in three subjects groups.

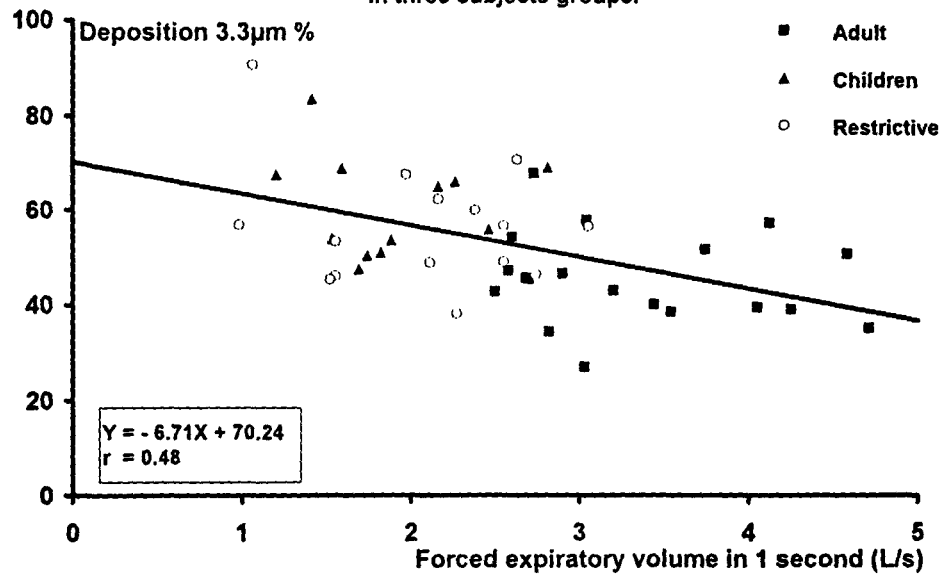


Table 1 : Individual main parameters observed during the incrementally graded exercise

Subjects	TR o/n	$\dot{V}$/V o/n	Max. Exercise		Minute ventilation				
			Watts	C. F.	Max (L)	Oral %	Rest (L)	Oral %	SP (L)
1		3.24	225	145	71.4	49.9	10.0	0	50
2	0.63	1.47	175	133	72.4	67	12.7	0	40
3	0.17	1.68	200	124	71.7	63.6	13.9	0	65
4	0.45	1.18	175	157	84.0	51	10.0	0	60
5	0.75	1.29	125	145	44.2	47	12.0	0	40
6			225	158	56.8	45.4	11.6	0	38
7	0.45	3.00	225	157	90.8	55	15.4	23	
8	0.13	3.33	175	136	50.4	57	8.3	10	
9		1.00	150	164	24.7	30	9.6	30	
10		2.30	125	121	22.1	34	12.2	17	

$\dot{V}$/V o/n = Flow / volume curve oral / nasal
TR o/n = Total resistance oral / nasal
C. F. = Cardiac frequency / minute
SP (L) = Nasal to oronasal ventilation switching point

Table 2 : Mean percent total depositions of inhaled particles in the airways of 4 groups of subjects.

Particle Size Deposition	1.2µm %	2.3µm %	3.3µm %
Healthy Non Smokers n = 18	26.1 (2.9)	34.2 (2.5)	45.2 (2.3)
Obstructives n = 15	39.2 ** (5.4)	47.7 ** (5.3)	56.7 (5.2)
Restrictives n = 15	37.7* (2.8)	46.3* (3.1)	56.6 * (3.3)
Children (8 - 15 y) n = 13	20.6 (5.2)	47.1 * (3.8)	60.3* (2.9)

Standard error on mean = (sem)
Wilcoxon non parametric test : * = $p \leq 0.05$ ** = $p \leq 0.02$

Head of project 4: Dr. Patrick

II. Objectives for the reporting period

(A) To conduct further studies on the clearance of particles deposited in the lung by alveolar microinjection, in order to improve our understanding of the mechanisms underlying alveolar clearance. From earlier studies with colloidal gold particles, it remained to determine the extent to which alveolar clearance depended on particle dissolution.

(B) To continue the study of the functional morphology of large airways, to explore the possibility that the delayed clearance of particles might be related to discontinuities in the muco-ciliary clearance process. Morphometric studies on rat trachea would be extended to other strains and species.

Aims for the next reporting period

The study of the functional morphology of the large airways will be concluded, with (i) data collection and analysis for additional species, and (ii) further analysis of the data in terms of the distribution of lengths of non-ciliated sections of tracheal epithelium.

New studies will be commenced in which, following intra-tracheal instillation of insoluble particles, the amount of material retained on the tracheal surface and in the tracheal wall is determined at sacrifice. The tracheal content will be analysed into fast-moving and stationary (or slow-moving) particles on the airway surface, as well as material incorporated into the tissue. The aim of these studies is to explore the mechanism(s) underlying the delayed tracheo-bronchial clearance of particles in man.

III. Progress achieved including publications

(A) Alveolar Clearance

In previously reported studies in the rat following the intra-alveolar microinjection of ^{195}Au-labelled colloidal gold particles, the clearance kinetics from the alveolar region were determined for 6 animals over 462 days. The overall clearance of ^{195}Au was found to be well described by two exponential terms, with 22% being cleared with a half-time of 14 days and the remainder with a half-time of 583 days. At 4 times during this study, the urinary and faecal excretion of ^{195}Au was measured, with the aim of analysing the overall rate of lung clearance into (i) particle transport up the conducting airways, and (ii) the absorption to blood of dissolved gold. The last two time points (273 and 462 days) were chosen such that only the very slow clearance term was still significant.

In order to provide necessary information about the excretion of dissolved gold from the blood circulation, a study was undertaken in which ionic gold chloride ($H^{195}AuCl_4$) was injected intravenously into 5 rats. Excretion was monitored over 3 weeks. Over this period 30% of the ^{195}Au was cleared from the body, 16.8 $\pm$ 0.9% appearing in the urine and 13.3 $\pm$ 0.7% in the faeces.

Assuming that ^{195}Au appearing in the blood from dissolved gold colloid was similarly partitioned between urine and faeces, the overall clearance in that study could be analysed as follows:

Days after microinjection	Proportion of overall clearance (%)	
	Particle transport	Absorption to blood
28	75.6	24.4
56	93.6	6.4
273	31.5	68.5
462	22.7	77.3

Thus at the later times, when only the slow phase of clearance remained, more ^{195}Au was cleared by dissolution than by particle transport up the bronchial tree. As expected, particle transport was more prominent at the earlier times.

After the injection of gold chloride, 42% of the amount injected was retained in the liver, with lesser amounts in the kidney and spleen, and only 0.25% in the lung. The extent of liver uptake raises the possibility that the gold chloride was converted into metallic gold in the blood. Before injection the solution was at pH 3.5 and did not precipitate. In contrast, in the intra-alveolar microinjection study with colloidal gold, the mean uptake of ^{195}Au by the liver had never exceeded 1.2% of the initial lung content.

Clearly caution needs to be exercised in applying the gold chloride excretion data to the excretion rates in the colloidal gold study. Nevertheless it appears that, even for such an insoluble material as metallic gold, long-term clearance from the alveolar region of the lung is at least partly the result of dissolution and absorption to blood. It is concluded that the rate of particle transport from the alveoli is known only approximately.

(B) Functional Morphology of the Conducting Airways

This study aims to see if delayed tracheo-bronchial clearance, and in particular the apparent differences in the extent of slow clearance between animals and man, can be explained in terms of the mucociliary clearance apparatus of the large airways.

Work has continued on the study by image analysis of the proportion of the epithelium of the trachea which is covered by cilia, extending the collection of morphometric data to other strains and species besides the Fischer F-344 rat. The circumference of the epithelial surface in transverse sections was determined, together with the sum of the segments of the circumference which are devoid of cilia. For the F-344 rats, sections from each animal were taken from 3 levels: proximal, middle and distal trachea. In all strains and species, each section was divided into 4 quadrants: dorsal, ventral and two lateral.

A statistical analysis of the F-344 rat data showed that there were no significant differences in the fraction of non-ciliated epithelium between the 4 quadrants, nor between the 3 levels; there were however differences between individual animals. Therefore only the middle portion of the tracheas from other strains and species was studied.

The HMT strain of rat gave similar results except that there was no significant difference between individual animals. A comparison between the two strains showed a statistically significant difference: the overall percentage of non-ciliated epithelium was $54.3 \pm 1.5\%$ in the F-344 rat, as against $66.7 \pm 2.8\%$ in the HMT strain.

Data have been collected for CBA mice and for Guinea pigs. In addition to transverse sections of trachea, some measurements are being made from longitudinal sections, to compare the discontinuities in ciliary cover in these two dimensions.

Further analysis of the data has commenced in terms of the distribution of the lengths of non-ciliated sections of tracheal epithelium. Here the working hypothesis is that if discontinuities in the ciliary cover of the epithelium do affect the rate of mucociliary clearance, and conceivably the extent of delayed clearance, then larger gaps between ciliated areas will have more significance for mucus flow than shorter intervals.

This analysis has been carried out to date for transverse sections of the trachea of two F-344 rats. Averaging over the 3 levels and 4 quadrants referred to above, the results are as follows:

Minimum length of non-ciliated epithelium scored (μm)	Percentage non-ciliated	
	Rat 1	Rat 2
0	0.57	0.67
2	0.57	0.67
4	0.56	0.66
6	0.54	0.64
8	0.51	0.62
10	0.49	0.60
15	0.44	0.54
20	0.37	0.48
25	0.31	0.42
30	0.26	0.38
35	0.23	0.34
40	0.19	0.31
45	0.17	0.29
50	0.15	0.25

It can be seen that while the total extent of non-ciliated epithelium (minimum length scored = 0) amounted to 57 and 67% of the total surface in these 2 rats, when only non-ciliated lengths greater than 50 μm were included, the fractions were still as high as 15 and 25%. This analysis is being extended to the tracheas of more animals and to the other species for which tissue sections are now available. It is hoped that this will give a better insight into species and strain differences than is gained by comparing only the overall fraction of non-ciliated epithelium.

Publications

Patrick G & Stirling C (1992), The transport of particles of colloidal gold within and from rat lung following local deposition by alveolar microinjection, *Environmental Health Perspectives* 97, 47-51.

Patrick G & Stirling C (in press), The redistribution of colloidal gold particles in rat lung following local deposition by alveolar microinjection, *Annals Occup Hygiene*.

Head of project 5: Dr. Stradling

II. Objectives for the reporting period

To complete the first phase of a collaborative study with CIEMAT on the radiological implications of inhaling ^{239}Pu and ^{241}Am present in two contaminated soil fractions obtained from Palomares. The soil for this work was collected from close to the site of impact of one of the nuclear bombs.

The next reporting period will involve biokinetic studies of ^{239}Pu and ^{241}Am present in respirable dust obtained from the total soil and the 20-40 μm fraction. In-vitro tests conducted at CIEMAT have shown that ^{241}Am is leached from the total soil to a much greater extent than ^{239}Pu, whilst the 20-40 μm fraction contains the highest abundance of ^{239}Pu (42%).

III. Progress achieved including publications

On January 17th 1966 an aviation accident occurred in the airspace over Palomares in the Spanish province of Almeria. As a consequence, four thermonuclear bombs fell to the ground and in two of them the weapon's high explosive component detonated., The aerosol produced by the accident resulted in the contamination of 226 hectares of mainly farmland and brushland. From the standpoint of potential risk to health, the most important radionuclides were plutonium-239+240 (referred to hereafter as ^{239}Pu) and americium-241. Despite extensive decontamination these actinides are still present in the soil.

Since the accident, comprehensive studies have been undertaken on the correlation between ^{239}Pu and ^{241}Am concentrations in the soils and their granulometric, mineralogical and chemical composition. This work is important for evaluating the potential radiological hazard associated with the dispersion of the actinides by resuspension, erosion and hydrological transport.

The principal route of intake of these actinides is considered to be by inhalation of contaminated dust particles. Assessments of the radiation doses to persons working or living in the vicinity of Palomares have utilised the data obtained from extensive measurements of their concentrations in air and in urine but assumptions have had to be made concerning their biokinetic behaviour in-vivo. The aim of the collaborative experiments with CIEMAT are designed to provide an experimental basis for improving the assessments of the committed effective dose.

In the first study the biokinetics of ^{239}Pu and ^{241}Am present in two respirable dusts derived from a chemically and mineralogically defined soil obtained from close to the site of impact of one of the bombs were investigated after their deposition in the rat lung. The first dust represented the natural respirable fraction of the soil (<5 μm); the second, of similar particle size, was obtained by grinding the soil fraction of particle size 125-250 μm, and known to contain the highest concentration of ^{239}Pu. Autoradiography showed that both fractions contained particulate and dispersed forms of these actinides.

Initially, the biokinetics of ^{239}Pu and ^{241}Am present in the fractions were investigated after their deposition in the rat lung. On the basis of the transfer rates of ^{239}Pu and ^{241}Am to blood, the respirable fraction of the soil was estimated to contain 10% of a class W compound and 90% of a class Y compound as defined by ICRP in publication 30; the biokinetics of these actinides in the ground fraction of the 125-250 μm component were compatible with a class Y compound. The committed effective doses for adults, 10y-old children and 1y-old infants inhaling 1 μm AMAD particles of the respirable fraction were calculated, on the basis of the 1990 ICRP recommendations, to be 0.064, 0.091 and 0.198 mSv Bq^{-1}. The corresponding Annual Limit on Intake for workers employed for any further decontamination measures is 310 Bq and the limits on intake for adult members of the public 16 Bq y^{-1}.

The biokinetics of ^{239}Pu and ^{241}Am in adults were predicted by combining the transfer rates to blood obtained from the animal experiments with the results of other studies in which the mechanical clearance rates of particles from the human lungs were obtained after volunteers had inhaled ^{85}Sr and ^{88}Y labelled fused clay particles. It was deduced that for the above dust fractions, the long-term lung retention half-times in adults would be about 600d and that the amounts of ^{239}Pu transferred to the blood from the pulmonary region over a period of 50y would be about 13% of the initial deposit. The predicted biokinetics of the ^{239}Pu and ^{241}Am in adults suggest that external monitoring of the chest could be used to advantage for assessing intakes incurred by workers, but that the technique would be of little value for inadvertent public exposure.

Publication

Stradling GN, Gray SA, Moody JC, Hodgson A, Ellender M, Phipps A, Pearce M, Wilson I, Iranzo CE, Rivas P, Espinosa A, Aragon A and Iranzo E. Biokinetics of ^{239}Pu and ^{241}Am in the rat after the pulmonary deposition of contaminated dust obtained from soil samples at Palomares: the implications for human exposure. NRPB Memorandum (in press).

Head of project 6: Dr Iranzo

II. Objectives for the reporting period

1. To provide data about the characteristics of the contaminated dusts in relation to particle size, chemical and mineralogical composition, and correlation of these characteristics with the Pu and Am concentrations and the physico-chemical forms of these actinides.

2. To make *in vitro* simulated pulmonary leaching experiments to assess the physiological dissolution characteristics of plutonium isotopes and americium present in dust samples.

Objectives for the next reporting period

1. To make *in vitro* simulated pulmonary leaching experiments on two respirable fractions, over a period of one year. In the first three months the solution will be changed every week, and afterwards the solution will be changed fortnightly.

2. To make simulated pulmonary leaching experiments using two samples of 10 mg each of (i) the respirable fraction and (ii) that between 125 and 250 μm (this fraction will be ground until over 90% of the particles are less than 5 μm).

3. After the sixth month of the experiment mentioned in objective (1), the physiological solution will be changed, for one of the samples, but maintaining the same experimental conditions.

III. Progress achieved including publications

Studies have been carried out on a defined soil contaminated as a result of the aviation accident at Palomares in January 1966. This soil was taken from an area close to the impact point of one of the bombs.

Particle size distribution analyses have been performed and the plutonium content has been determined in the different fractions. The highest activity concentration was determined in the size fraction between 125 and 250 μm. This fraction showed a value of 6389 ± 315 Bq g^{-1}.

Because of this high value, this fraction was selected for administration to rats. It was first ground to obtain a size fraction < 5 μm. The specific activity of the respirable component of this soil fraction was about 2000 Bq g^{-1}, lower than the activity of the parent material.

The dust representing the natural respirable fraction of the soil, *ie.* the fraction <5 μm, was also selected for administration to animals. The plutonium content of this fraction was about 900 Bq g^{-1}.

Autoradiographic studies showed that both fractions contained both dispersed forms and particulates. It appears that each form represents about one half of the total activity

present.

Chemical and mineralogical studies showed that both fractions consisted mainly of SiO_2, Al_2O_3, CaO, Fe_2O_3 and CO_3^{2-}. The main mineralogical components are quartz, plagioclase and dolomite. The amounts of quartz are very different in the two fractions, being 10% in the particle size fraction <5 μm and 60% in the particle size fraction between 125 and 250 μm. The calcite content also differs considerably between the two fractions, being 58% and 8% respectively.

Experimental method for pulmonary leaching

For pulmonary simulation three small amounts of respirable fraction dust were used. Each one was placed in a tube-shaped, semipermeable membrane closed at both ends. Each membrane was placed in a container with 400 ml of pH 7.3 physiological solution. All the containers were kept at 37°C and were shaken for half an hour every three hours to simulate the physiological conditions of the lung. Each week the solution was replaced by another which had been recently prepared, and the solution removed was analysed using radiochemical techniques.

Results

Preliminary results are shown in the following figures.

Conclusions

Under the conditions adopted in this first experiment, it is possible to conclude that:

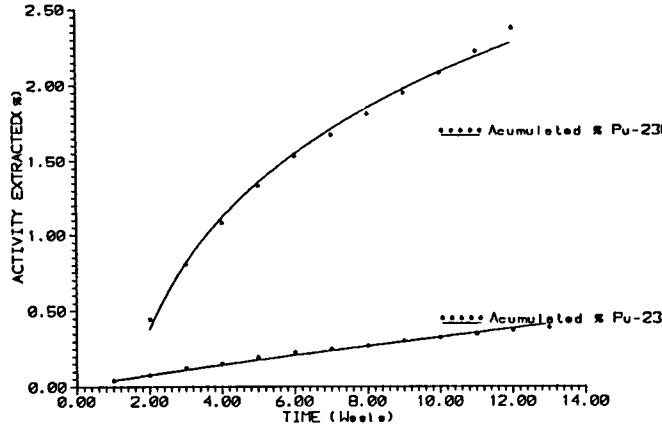
- The first preliminary results show different behaviour for the plutonium isotopes. Plutonium-238 presents a higher leaching rate during the first weeks, decreasing to approximately one-fifth of that initially extracted towards the end of the third month.

- The percentage of plutonium-239 leaching during the 13 weeks varies slightly, the total extracted plutonium being less than 0.5% of the plutonium contained in the sample.

- The weekly extracted percentage of $^{239}Pu/^{238}Pu$ ranges from 0.07 to 0.16 throughout the experiment.

- The cumulative percentage of $^{238}Pu/^{239}Pu$ extracted in three months is greater than 5.

These preliminary data are not enough to establish definitive conclusions. The next experiments, under different conditions, will or will not confirm this behaviour of the plutonium isotopes.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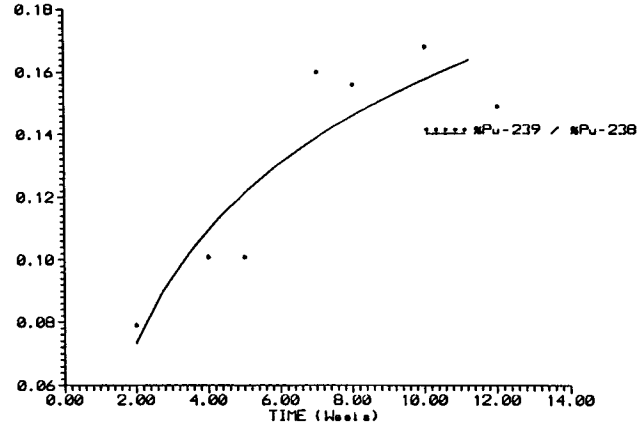
Acumulated % Pu-238
Acumulated % Pu-239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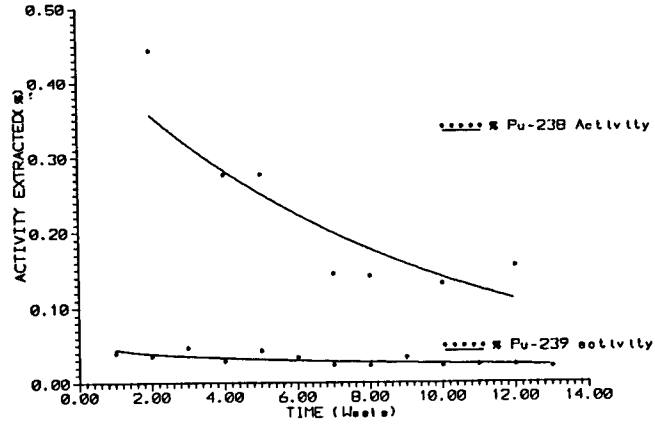
ACTIVITY EXTRACTED(%)
0.50
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4.00
6.00
8.00
10.00
12.00
14.00
TIME (Weeks)
% Pu-238 Activity
% Pu-239 activity

List of publications

E Iranzo, S Salvador and C E Iranzo. Air concentrations of ^{239}Pu and ^{240}Pu potential radiation doses to persons living near Pu-contaminated areas in Palomares, Spain. Health Physics, 52, 4, 453-461, USA, (1987).

E Iranzo, E Mingarro, S Salvador, C E Iranzo and P Rivas. Geochemical distribution of plutonium and americium in Palomares soil. Cycling of long-lived radionuclides in the biosphere. Observations and models 2, 392-419, Madrid, (1987).

E Iranzo, A Espinosa and C E Iranzo. Evaluation of remedial actions taken in an agricultural area contaminated by transuranides. Impact des accidents d'origine nucleaire sur l'enviroment, 2, F1-F21, Cadarache, France, ISBN 2-7272-0143-5, (1988).

E Iranzo, P Rivas, E Mingarro, C Marin, A Espinosa and C E Iranzo. Distribution and migration of plutonium in soils of an accidentally contaminated environment. Radiochimica Acta 52/53, 249-256 (1991).

C Iranzo and A Espinosa. Problems derived from the application of the new ICRP recommendations in internal dosimetry. Iª Conferencia internacional sobre: "Las Implicaciones de las Nuevas Recomendaciones de la C.I.P.R. en las Practicas e Intervenciones de la Proteccion Radiologica y IV Congreso Nactional de la Sociedad Espanola de Proteccion Radiologica. Salamanca, Espana (Novienbre 1991).

J A B Gibson, A Birchall, R K Bull, K Henrich, E Iranzo, D J Lord, J Piechowski, E Sollet, N P Tancock and C Wernli. A European intercomparison of methods used for the assessment of intakes of internally deposited radionuclides. Radiation Protection Dosimetry, Vol. 40, No. 4, pp 245-257 (1992).

A Garcia-Olivares and C E Iranzo. Resuspension and transport of plutonium in the Palomares area. Journal of Environmental Radioactivity. July 1992.

Progress Report

Contract: FI3P-CT920003 **Sector:** A2A

Title: Promotion of formation, knowledge and exchange of information in radioecology.

1) Myttenaere UIR

I. Summary of Project Global Objectives and Achievements

IUR is dedicated to promote the cause of radioecology, the study of the relationship between radioactivity and the living organisms, with a view to protect Man from the harmful effects of radionuclides present in the environment. Continuous research is needed to maintain progress in this important area of environmental sciences.

One of the main objectives of IUR is to encourage the exchange of informations and expertise in the field of radioecology, particularly in case of major accidental releases of radioactive material, such as the Chernobyl accident. It must be emphasised the role played by IUR in the promotion of cooperation between CIS and Western scientists. This cooperation and exchange of informations stimulate interactions that increase our understanding of radioecology problems.

Another major objective aims to promote the formation and training of young scientists in order to develop interest for radioecology among them. Training courses on specialized radioecological topics are foreseen as well as the development of a curriculum for a textbook on Radioecology.

Achievments:

The IUR activities were developed in two main directions:

- the first one is based on the Steering Committees work which were established to support specific activities such as, i.e., Summer schools (SC1), Publications (SC2), Cooperation with the Central and Eastern countries (SC3). The SC2 is charged with the quarterly Newsletter and prepares a textbook on Radioecology which is expected to be issued in 1994;
- the second one is based on several Task-forces dealing with different problems which ask for some updating in radioecology to follow the evolution of the needs for knowledge and efficiency. The detailed objectives and progress achieved by each particular Task-Force during the reporting period 1/9/92 to 31/8/93 are given under II and III.

Head of project 1: Prof. Myttenaere

II. Objectives for the reporting period

Implementation of the objectives of the following Task-Forces :

1- Priorities in Radioecology.
2- Assessment of the relative dose contribution of various pathways under different environmental conditions, including semi-natural environments.
3- Assessment of the relative importance of various freshwater exposure pathways and the major parameters influencing pathways.
4- The Behaviour of less commonly considered radionuclides
5- The effects of radiations on organisms in their natural environment

Objective for the next reporting period : 1-9-93 to 31-5-94

TF1 At the IUR annual meeting in October 1993 the draft report mentioned below will be discussed, commented and completed.

TF2 A meeting of the nucleus group of this task force is scheduled for mid September 1993 in order to summarise the conclusions from the first series of written abstracts and to define the focal points for the second study campaign.

TF3 The detailed work programme of this task group will be set up on hand of the results of the Lisbonne Seminar in March 1994 on freshwater and estuarine radioecology, where all relevant exposure pathways will be covered.

TF4 From the above data the task force will identify the possible radioecological relevance of these radionuclides. A report is under preparation and will be discussed during the IUR meeting at Udine in October 1993.

TF5 At the IUR annual meeting in October 1993 the task force will meet to refine the criteria for the selection of relevant literature and an examination will be made of any available bibliographies and review articles to determine the relevance of the papers. Decisions will also be made concerning the most cost-effective way of proceeding.

III. Progress achieved including publications

Task-Force 1 : Priorities in Radioecology

The draft report covers the following subjects:

- global sources of radioactivity
- pathways for radionuclide transport
- accidental conditions, countermeasures and rehabilitation
- fundamental mechanisms involved in radionuclide transport

At the end of each chapter the topics for radioecological research targetted towards future conditions are listed; a last chapter is devoted to the general conclusions drawn on the basis of the information presented in the previous chapters.

Task-Force 2 : Assessment of the relative dose contribution of various pathways under different environmental conditions, including semi-natural environments.

The first analyses show that it is necessary to differentiate between an initial phase of primary deposition of radioactivity and superficial contamination of surfaces and vegetation, when the partitioning of dose contributions is mainly determined by the relative abundance of the radionuclides in the incoming air masses, climatic/seasonal conditions, land use as well as living habits and the subsequent phases, when the time courses of dose contributions are additionally

governed by greatly varying and partly counteracting natural processes and parameters like decay, physical and biological dilution, bioaccumulation, bioavailability, ecological half-lives, migration and redistribution.
While the mechanisms responsible for the relative importance of exposure pathways in the initial phase are generally well understood, predictions become less reliable for subsequent phases especially for non-agricultural areas (semi-natural, urban), which for certain pathways have been identified as "critical areas". The combination of those regional conditions with non-average living habits leads to some exceptional exposures, not only in absolute but also in relative values of the various contributions.
Very drastic shifts of the relative importance of exposure pathways may be induced by countermeasures which superimpose the natural processes. Undoubtedly, such actions may have great short and medium-term effects on the total and relative dose contributions to individuals, however, our knowledge about long-term and collective dose shifts is limited. It is interesting to note that dose predictions may not only fail because of insufficient understanding or modeling of radioecological processes but also because of lack of statistical evaluations, e.g. on dietary, occupational and recreational habits of the average and non-average (critical) population.

Task-Force 3 : Assessment of the relative importance of various freshwater exposure pathways and the major parameters influencing pathways.
TF3 is setting up a list of all laboratories working in the field of aquatic radioecology indicating the principal ongoing activities. A correspondence network has been established with some of these laboratories.
The main work of this Task Force will, however, start after the Lisbonne Seminar.

Task-Force 4 : The Behaviour of less commonly considered radionuclides
This Task-Force met at Riso on 17-18 November 1992 where it identified 35 radionuclides which are less commonly studied in radioecology. Each radionuclide has been listed and completed by its physical characteristics (sources, half-life, decay scheme, etc.), environmental parameters (concentrations), radioecological parameters (Kd, CF), radiobiological parameters (ALI), and specific properties and problems.

Task-Force 5 : The effects of radiations on organisms in their natural environment
In consultation with the members of this task force, a strategy has been developed to commence the assimilation of the experience gained in the former Soviet Union into a comprehensive account of the potential effects of increased radiation exposure on populations of wild organisms. The input should come from environments which have been accidentally contaminated to the extent that dose rates are sufficiently high that there may be clearly detectable effects in organisms which might also be expressed at the population level.

Publications :

1 Résumés détaillés des communications - Kiev 1991
2 Proceedings of I.U.R. Soviet Branch Seminar 1991
3 Proceedings Znojmo, October 1992
4 Activity reports from the IUR Working Groups IUR Pub R-9211-01
5 Report of the Working Group Soil-to-Plant Transfer Factors, Madrid 1992 IUR Pub R-9212-02
6 IUR Newsletter (n° 6-7-8-9)

Progress Report

Contract: FI3P CT920029 Sector: A21

Title: Towards a functional model of radionuclide transport in freshwaters.

1)	Hilton	NERC
2)	Ortins	LNETI
3)	Cremers	Univ. Leuven (KUL)
4)	Foulquier	CEA - Cadarache
6)	Sansone	ENEA
7)	Vanderborght	Univ. Antwerpen
8)	Fernandez	Univ. Malaga
10)	Comans	ECN

I. Summary of Project Global Objectives and Achievements

Work programmes are proceeding according to plan. It is too early to make an assessment of the effect of likely results on the movement towards a functional model. However, the final report at the end of next year will incude an assessment of the extent to which this programme has developed new ideas for incorporation into a functional model and identify gaps requiring further work.

Head of project 1: Dr. J. Hilton

II. Objectives for the reporting period

1) To measure the ammonia concentration in epilimnetic and hypolimnetic sediment pore water of a lake through a biological season in order to estimate the effect on the Cs-137 K_d.
2)to explore potential methods for the measurement of boundary layer thickness in-situ.
3) to assist in the collection of a large number of cores from Esthwaite water, in conjunction with Dr. Comans (ECN), in order to measure in-situ K_d.

III. Progress achieved including publications

Background.

Attempts to model the removal of radioactivity from the water column after Chernobyl have resulted in an ambiguity. Work in some lakes, particularly that by Hesslein and co-workers, show that the observed rate of removal of radiocaesium from some Canadian lakes can only be achieved by the inclusion of a process of direct diffusion across a boundary layer. However, studies on European lakes by Robbins et al., and Davison et al. over estimate the rate of removal if this process is included.

The rate of loss of material from the mixed water layer is given by Fick's law:

$$V\frac{dC}{dt} = \frac{DA(C-C_s)}{z}$$

where:
V is the volume of the perfectly mixed body of water;
D is the molecular diffusion coefficient of the appropriate radioactive ion in water,

(see Li and Gregory (1974) for typical values of D);
A is the surface area of the sediments in contact with the mixed water;
z is the boundary layer thickness;
C is the concentration of dissolved radionuclide in the mixed water column;
C_s is the radionuclide concentration in the sediment pore water.

The rate of transfer would be significantly reduced if, either the boundary layer were much thicker in the two European lakes than in the Canadian study, or, the radiocaesium concentration in the sediment pore water were much greater than the zero level assumed by Hesslein et al. Unfortunately very little information is available on boundary layer thicknesses in lakes, or on the variability of ammonium in sediments - the key parameter defining the pore water concentration of radiocaesium. The work in this programme is designed to quantify these two factors in some of the European lakes in an attempt to identify the causes of this anomaly.

1) In order to quantify the importance of a non-zero concentration of radiocaesium in the pore water, replicate samples have been taken from one site in deep water and one site in shallow water on eight occasions, to date, throughout the year. At the same time single samples were taken along a transect of varying depth across the lake. Each core was sliced at 0.5cm intervals down to 2.5 cm, the slice was then split into three and the pore water extracted from each section by centrifugation. Ammonia was measured by colorimetry so as to obtain a) an estimate of the ammonia concentration in the surface pore water and b) a corroboration that this value was not significantly enhanced by localised decomposition at the sediment water interface. These data will provide estimates of the within slice variation, the variation between cores at a single place and time, the variation between sites at a single time and the variation with time of all these.

By using the K_d v ammonia plot developed by Comans et al (in preparation) it will be possible to estimate the change in pore water Cs-137 concentration with time and space after the Chernobyl event. These data will then be used to assess i) the likelihood that localised ammonia generation in the sediment could be a significant factor in the inhomogeneity observed in sediment core inventories taken at the same place at different times; i) the likelihood that non zero radiocaesium concentrations in the pore water in hypolimnetic sediments reduce the importance of transport across the boundary layer in deep water compared to shallow water.

2) In lakes and rivers, the boundary layer is a very diffuse region within which the turbulence in the open water reduces to zero at the sediment surface. It may be up to 50 cm thick but is highly variable and difficult to model as it includes both an advective component and a diffusive component. In model systems this diffuse layer is replaced by a much thinner layer within which only diffusive transport is allowed. Although a crude approximation it has been used successfully in estimates of fluxes of minerals in manganese nodule formation in

the oceans. It is difficult to measure this boundary layer thickness but a method which has been used successfully is to place on the sediment gypsum rock samples which have been prepared so as to expose one flat surface to the water. The loss of gypsum determined after a known deployment time gives an estimate of the diffusive flux across the boundary. Since the solubility product of gypsum is known, the boundary layer thickness can be estimated from the rate of dissolution.
Unfortunately the preparation of gypsum rock is difficult because of the large amounts of dust produced when sawing and, the rock is very soft so that it often breaks during the preparation. In order to circumvent these problems we have modified a method of using cast blocks of gypsum as a dissolution source. Starting from plaster of paris reproducible surfaces can be produced much more easily than with sawn rock. Because the cast shape is consistent with time it has been possible to design and build a practical apparatus to deploy the block at the sediment water interface. Some problems were originally experienced due to the material crumbling when exposed to water. However we have now overcome this problem by more strict control of the production procedure and intend to make some initial measurements in the near future.

3) 12 cores were collected in August 1992 from Estwaite water. A previous expedition had shown that, contrary to expectation, radiocaesium concentrations in the pore water were at, or below the limit of detection so that counting errors on these samples were high. As a result, K_d estimates were not as precise as hoped for in the study. By increasing the diameter of the cores to 10 cm and increasing the number of cores which were extruded and processesed under nitrogen in a glove box about a litre of pore water was obtained for measurement. THis has allowed in-situ K_ds to be measured down the core and correlated with ammonium concentrations in the pore water. More details of this work are given by Dr. Comans.

References:

Hesslein, RH., WS.Broecker and DW.Schindler. (1980) Fates of metal radiotracers added to a whole lake: sediment-water interactions. Can. J. Fish Aquat. Sci. v.37, 378-386.

Robbins, J.A., G.Lindner, W.Pfeiffer, J.Kleiner, H.H.Stabel and P.Frenzel. (1992) Epilimnetic scavenging of Chernobyl radionuclides in Lake Constance. Geochim. cosmochim. Acta. v.56, pp 2339-2361.

Davison, W., J.Hilton, J.Hamilton-Taylor, F.Livens and M.Kelly. (1993) Measurement, interpretation and modelling of Cs-137 transport through two freshwater lakes after Chernobyl. J. Environ. Radioactivity. v.19; 125-153.

Publications from work carried out under the EC programme:

Hilton, J., R.S.Cambray, and N.Green. (1992) Chemical fractionation of radioactive caesium in airborne particles containing bomb fallout, Chernobyl fallout and atmospheric material from the Sellafield site. J. Environ. Radioactivity. v.15; 103-111.

Hilton,J., F.Livens, P.Spezzano, and P.Leonard. (1993) The retention of radioactive caesium by different soils in the catchment of a small lake. Sci. Tot. Environ. v.129; 253-266.

Davison, W., J.Hilton, J.Hamilton-Taylor, F.Livens and M.Kelly. (1993) Measurement, interpretation and modelling of Cs-137 transport through two freshwater lakes after Chernobyl. J. Environ. Radioactivity. v.19; 125-153.

Scientific Responsable of Project 2: Dr. A. Ortins Bettencourt
Head of Project 2: Dr. Maria Carolina Vaz Carreiro

II. Objectives for the reporting period

1) Sorption-desorption processes of radionuclides in sediments

To improve the understanding of sorption-desorption dynamics of several radionuclides, namely ^{137}Cs and ^{90}Sr, in sediments from Fratel Reservoir (Tejo River).

2) Biological processes

To improve the evaluation of the reliability of transfer factors in freshwater trophic chains (Fratel Reservoir), mainly through the knowledge of parameters affecting their variability.

III. Progress achieved

1) Sorption-desorption processes of radionuclides in sediments

It is demonstrated that, on the basis of a desorption methodology, corresponding to infinite bath boundary conditions, well-defined desorption plateu values can be generated for radiocaesium. It is shown that such desorption levels, obtained in very low background concentrations of K and NH_4 (10^{-3}M), are significantly higher than those obtained by classical procedures based on the use of very concentrated solutions (1M). In all cases, significant fixation levels can be demonstrated within very short time intervals (days) after contamination, followed by a slow increase in fixation with time. At this stage, the dynamics of these aging processes could not be quantified and we lack the quantitative diagnostic criteria for predicting fixation levels on the basis of readily measurable properties of the systems. However it appears that the cationic composition of the liquid phase (and therefore the solid) plays a key role in the fixation behaviour: strongly hydrated cations (Ca, Mg, Na) lead to a pronounced enhancement of radiocaesium fixation in the solid phases; poorly hydrated ions such as K and NH_4 have the opposite effect and promote sorption reversibility. Drying a sediment at room

temperature has a very limited effect on fixation but appears to put a brake on the aging effects.

Still, under the reported objectives, a selective and sequential ion exchange separation of radiostrontium and radiocesium, with a previous characterization of the sediments for radiostrontium, was carried out.

The comparative study of desorption behaviour of radiostrontium and radiocesium, using a combination of infinite bath boundary methods, concentrated solutions of potassium and ammonium, and solutions of cations characterized by high adsorption affinity for the sites of the regular ion exchange complex, demonstrate clearly that radiocesium and radiostrontium are associated with different regions of the solid phase. Radiocesium is quantitatively associated with the FES and its adsorption is only partly reversible; radiostrontium is quantitatively associated with the regular ion exchange complex and its adsorption is completely reversible. Such difference in solid-phase speciation is clearly demonstrated by a simple methodology allowing nearly complete separation of the ion-exchangeable forms of the two radionuclides.

These studies were develloped at the University of Leuven (Laboratory of Colloid Chemistry) by M.J.Madruga, who have been preparing a doctoral thesis. All the work concerning the participation of INETI on the Project, during the last year, is included and described on the Report of the K.U. Leuven.

2) Biological processes

Under the hypothesis that the variability in ^{137}Cs concentration factors (CF) in freshwater fish is influenced not only by potassium concentration in water, but also by the ratio potassium/stable cesium, some experiments were undertaken.

The main cation concentrations in Fratel Reservoir water, considering the means of three years are in Table 1.

Table 1 - Main cations in Fratel water

Element Concentration (ppm)				
Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	Cs^{+}
36±5	11±1	25±4	3.3±0.3	$(5.8\pm1.7)10^{-5}$

Artificial water was prepared with the above referred concentrations of Ca^{2+}, Mg^{2+} and Na^{+} and three different K^{+} concentrations: 0.35, 3.5 and 35 ppm. Cs^{+} concentration used was ten times higher than usual, 5.8×10^{-4} ppm.

Fish species was Chondrostoma polylepis polylepis, a Cyprinid, the most common species at Fratel Reservoir.

Groups of ten fishes, weighing about 1 g at the begining of the experiment, were kept in aquaria, with 5 l of the above referred water and with a ^{134}Cs concentration of about 15 Bq ml^{-1}, having had a previous acclimatization period of two weeks.

The water was changed every week and K^{+} concentration controlled.

Results showed that there was a quite good acclimatization of fish to the different K^{+} concentrations, even to the highest one. There was a significant increase in the ^{134}Cs accumulation in fish at the lowest K^{+} concentration, Fig.1. At the highest K^{+} concentration the ^{134}Cs accumulation is lower, but not so different from the control one (3.5 ppm) as it could be expected.

All the curves may fit to a power function as follows:

$CF = 6.6\ t^{0.81}$ $r^2 = 0.92$ ($[K^+] = 0.35$ ppm)

$CF = 0.77\ t^{1.06}$ $r^2 = 0.98$ ($[K^+] = 3.5$ ppm)

$CF = 0.37\ t^{1.15}$ $r^2 = 0.99$ ($[K^+] = 35$ ppm)

Taking into account these expressions, at time t=28 days, ^{134}Cs CFs for fish at the different K^+ concentrations in water are shown in Fig.2.

The uptake experiment was followed by the study of the ^{134}Cs loss by fish, keeping the same conditions, i.e., the same cation concentrations in water. The water was also changed every week, but every day a partial filtering to collect faeces was done, in order to a weekly evaluation of the loss through faeces or to the solution.

Results are not very different, Fig.3, however the ^{134}Cs loss by fish in the water with 0.35 ppm of K^+ is slower than in the water with the other two concentrations. A certain agreement between uptake and loss is observed.

Another kind of experiments is being carried out, reversing the K^+ and Cs^+ concentrations in water, i.e., using K^+ at the constant concentration of 5.8×10^{-4} ppm and Cs^+ at the following concentrations: 0.35, 3.5 and 35 ppm. All the other conditions are the same, including the two weeks acclimatization period. However results are not yet available.

Whole data are still under study and some other experiments are needed in an attempt to correlate the influence of stable cesium and potassium on the radiocesium accumulation.

It is necessary to stress that José A.G. Corisco, was absent from the laboratory, during the last three months of 1992, for a training at the CEN, Cadarache, Service d'Études et Recherches sur l'Environment.

Publications:

- Maria José B. Madruga. Adsorption-desorption behaviour of radiocesium and radiostrontium in sediments. (Thesis to be presented at the Katholieke Universiteit Leuven, Center for Surface and Colloid Chemistry, in October 1993).

- José Alberto G. Corisco, Maria Carolina Vaz Carreiro. Potassium-stable cesium balance on the radiocesium uptake and loss by the Cyprinid, Chondrostoma polylepis polylepis (to be presented at the International Seminar on Freshwater and Estuarine Radioecology, Lisbon, 21-25 March 1994).

IV. Objectives for the next reporting period

1) Sorption-desorption processes of radionuclides in sediments

Implementation in Portugal at the DGA/DPSR of the techinques for the study of sorption-desorption processes in sediments, which Maria José Madruga learned at the K.U. Leuven, with Prof. Cremers.

2) Biological processes

The whole set of experiments considered necessary to understand the influence of the balance of potassium-stable cesium on the accumulation of radiocesium by a freshwater fish, should be finished. Namely it is necessary to make experiments in the complete absence of potassium, but with stable cesium and the inverse situation.

Fig. 1 – ^{134}Cs uptake by C. **polylepis** from water with different K^+ concentrations

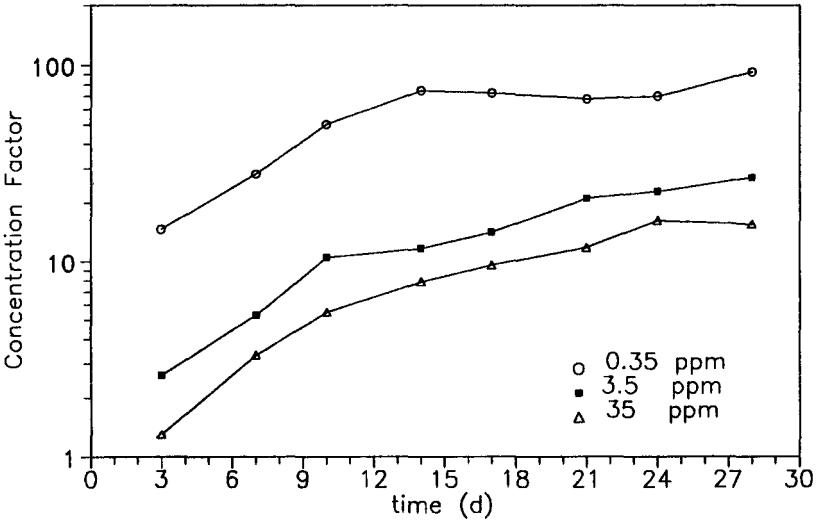


Fig. 2 – ^{134}Cs Concentration Factor in C. **polylepis** after 4 weeks of uptake from water with 0.35, 3.5 and 35 ppm K^+ concentrations.

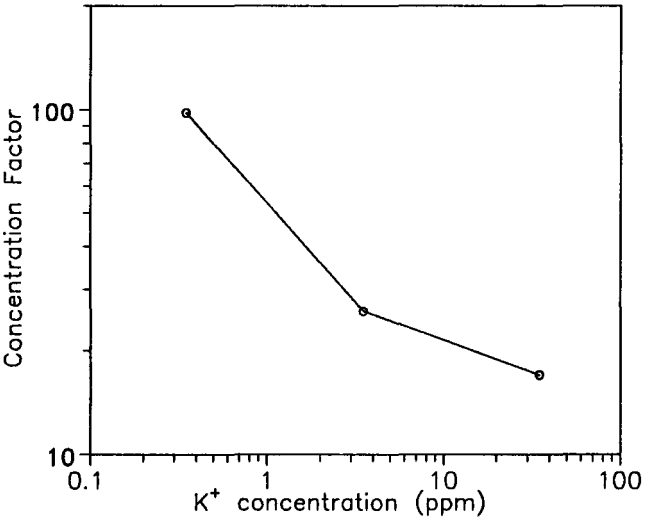
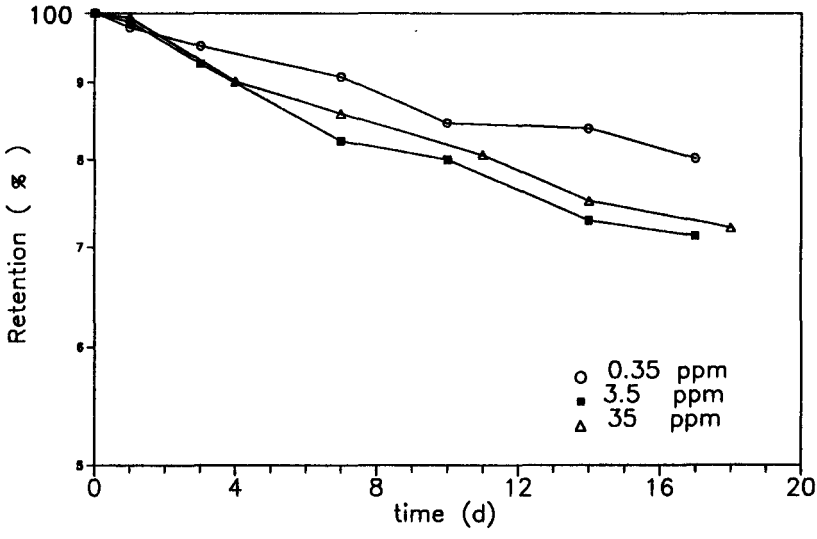


Fig. 3 – ^{134}Cs retention in C. **polylepis**, after 4 weeks of uptake from water with different K^+ concentrations



Head of project 3: Prof. Cremers

II. Objectives for the reporting period

1. The solid phase speciation of radiocaesium and its fixation behaviour in sediments

2. Aging effects of radiocaesium in sediments

3. Sorption-desorption behaviour of radiostrontium in sediments

III. Progress achieved including publications

1. The solid-phase speciation of radiocaesium

The behaviour dynamics (sorption-desorption) of radiocaesium in sediments is undoubtedly connected with its speciation in the solid phase. If we wish to make progress in our aim to develop diagnostic tools for making predictions of the fixation behaviour on the basis of readily measurable properties of the system (one of the objectives of our contribution) it is mandatory to acquire basic knowledge of the specific sorption properties in the sorption sites responsibles for the retention of radiocaesium

In the past, a number of arguments were developed for demonstrating that radiocaesium interception is essentially confined to the so-called Frayed Edge Sites (FES) of the micaceous minerals, present in the sediment. In essence, the arguments were based on two points: (a) a comparison of the specific interception potential of the

FES and the interception potential of the (reversible part of) exchange complex; (b) the failure of bulky cations to displace adsorbed radiocaesium. Since the quantitative characterization of the specific sorption potential was based on the use of masking techniques, thus eliminating the potential contribution of the regular exchange complex (REC), some doubt may remain as to the actual field behaviour.

The difference in sorption properties of the FES and REC manifests itself most convincingly through selectivity differences between ammonium and potassium ions in the FES; in these sites, sorption is significantly in favour of NH_4, the selectivity coefficient being generally around 5 to 7 (as obtained from masking techniques). In the REC (mineral and organic) the NH_4 to K selectivity coefficient is unity. Therefore, the most direct demonstration of the nature of the solid-phase speciation is to measure the solid/liquid partitioning response of radiocaesium in varying NH_4-K scenarios. The followwing experimental protocol was developed. Systems are thoroughly preequilibrated with mixtures of Ca (100mM) and K (10mM) containing varying levels of NH_4 (up to 5 mM) and K_D(Cs) is measured (24 hrs end-over-end shaking) for all scenarios. The Potassium Adsorption Ratios (PAR defined as $(m_K + m_N)/\sqrt{m_{Ca}}$) vary between 1 (Ca+K) and 1.5 (Ca + K + N), i.e. values exceeding those of fresh water scenarios (K=0.1mM, Ca+Mg = 1mM) by a factor of 10 at least. Therefore, the potential contribution of the REC in this experimental protocol is amplified by a factor of 10, as compared to freshwater conditions.

Equations were developed for predicting S/L partitioning. If radiocaesium is exclusively confined to the FES, we obtain:

$$\frac{(K_D.m_K)}{K_D.m_K} = 1+K_c(N/K)\ m_N/m_K \qquad (1)$$

in which $(K_D.m_K)$ refers to the $K_D^{Cs}.m_K$ product in the Ca-K scenario, $K_D.m_K$ to that product in the Ca-K-NH_4 scenario, $K_c(N/K)$ the NH_4-to-K selectivity coefficient in the FES and m_N/m_K the ratio of concentrations in the liquid phase. For a mixed behaviour, involving the action of both FES and REC, we obtain

$$\frac{(K_D.m_K)}{K_D.m_K} = \frac{1 + R}{(1+K_c m_N/M_K)^{-1} + R} \qquad (2)$$

in which R refers to the ratio of interception potentials of REC and FES. When the contribution of the FES vanishes, the $(K_D.m_K)/K_D m_N$ remains unity and is independent of m_N/m_K.

Figure 1 shows the results for illite clay and a set of freshwater sediments. It is seen that for illite the behaviour corresponds to the one predicted by eqn(1), i.e. a straight line relationship with $K_c(N/K) = 7$. For all sediments (including Devoke with an organic matter content of 30%) the behaviour is consistent with $K_c(N/K)$ values in the range of about 5 to 10. However, the slope increases at lower NH_4 concentrations, thus indicating some site heterogeneity in the FES. Consequently, it would appear that, in view

of the very significant response of $K_D(Cs)$ to the addition of NH_4 , radiocaesium is quantitatively adsorbed in the FES.

2. Fixation of radiocaesium and aging effects

Studies of radiocaesium fixation and the various factors affecting such process have been continued and are in line with conclusions formulated in our 1990-92 final report. However, some new and significant elements have been added. It was confirmed that, within a short time interval (days) after contamination, a rapid fixation manifests itself but the pattern is very erratic (for conditions corresponding to quantitative interception in the FES: see section 1) in that the size of such fraction may vary between some 10 to 50%. With time, the level of fixation increases but, on a laboratory time scale, the extent of these changes becomes difficult to demonstrate experimentally after some 3-4 months.

The system behaviour can be described in terms of a three box model comprising a compartment characterized by an instantaneous release behaviour, a compartment characterized by a slow release behaviour which can be characterized in terms of rate constants, and a no-release compartment. The systems is illustrated in figure 2. So far, neither the rapid fixation process, nor the dynamics could be rationalized or predicted on the basis of other measurable properties of the system.

Particular attention has been given to the effect of different ionic scenarios on fixation behaviour. The favourable effect of strongly hydrated ions (Ca, Mg: homoionic scenarios) on radiocaesium fixation was further confirmed in the case of sodium: these effects, although again quite erratic for different systems, are particularly pronounced within a very short time scale after contamination. With time, aging effects become less pronounced for Ca, Mg and Na-scenarios. These effects have been further confirmed by fixation studies in mixed ionic scenarios (varying PAR values): it was found that at increasing PAR (a range of 0.1-2), fixation tendency is significantly reduced. The fact that these effects manifest themselves for scenarios in which strongly hydrated ions are added to the system before or after radiocaesium contamination indicate that such ions affect the structural properties of the FES, leading to a partial occlusion of radiocaesium within the structure. The infinite bath boundary method was also applied to study radiocaesium desorption in a marine environment. Results are consistent with those obtained in a 10^{-3}M NH_4 or K protocol.

Particular attention was also given to the effect of drying on radiocaesium fixation, i.e. a rather important question in regard to sampling methodology and the subsequent assessment of radiocaesium availability. It was demonstrated that, provided drying is performed at room temperature, fixation is not altered. In fact, it was shown, on the basis of aging studies of air-dried samples, that the aging process is being halted and that the desorption levels provide a record, referring to the date of sampling.

3. Sorption-desorption behaviour of radiostrontium

A comparison study was made of the desorption behaviour of radiostrontium and radiocaesium using a combination of infinite bath boundary methods (as described in our 1990-92 final report), concentrated solutions of K, NH_4, Mg, Ca, Sr and solutions of bulky cations characterized by high adsorption affinity for the sites of the REC. The results of this study clearly demonstrate that radiostrontium and radiocaesium are associated with different regions in the solid phase: radiostrontium is quantitatively associated with the REC and its adsorption is completely reversible; radiocaesium is associated with the FES and its adsorption is only partly reversible. Such difference in solid-phase speciation of the two radionuclieds is demonstrated by the results of a methodology allowing a nearly complete separation of their ion exchangeable fractions.

The protocol is as follows:(a) double-labelled sediment samples which were aged for three days are submitted to a desorption treatment by dispersion in a 1N $SrCl_2$ solution (24 hrs) and the desorption levels are monitored by a combination of LSC and γ counting of the liquid phase; (b) subsequent to the Sr treatment, sediments are submitted to an infinite bath boundary desorption protocol (10^{-3}M NH_4 with Giese granulate: see 1990-92 report); (c) in parallel, single-labelled sediments (Sr-90 and Cs-137) are submitted to resp. a 1N $SrCl_2$ (Sr-90) and an infintie bath desorption scenario. The results of this study, carried out on six sediments are shown in Table I.

Table . Radiostrontium and radiocaesium desorption yields (%) for a set of sediments, in the presence of 1N SrCl2 and 10^{-3}M NH_4Cl Giese "(infinite bath" method)

Sediment	Double-labelled			Single-labelled	
	1N $SrCl_2$		10^{-3}M NH_4 Giese	1N $SrCl_2$	10^{-3}M NH_4 Giese
	^{90}Sr	^{137}Cs	^{137}Cs	^{90}Sr	^{137}Cs
T1	98.4	2.7	33.3	95.6	49.3
T2	99.3	3.3	28.2	97.3	46.5
A	96.4	1.3	39.6	96.8	61.9
S	97.1	1.2	40.2	97.3	67.5
D	99.7	3.2	65.7	99.0	91.7
Kr	100	1.1	16.6	97.9	21.8

T1 and T2: Tejo river; A and S: Tejo river estuary; D: Devoke reservoir; K: Kiev reservoir

It is seen that desorption levels for Sr-90 (double and single-labelled) in 1N $SrCl_2$ vary between 96 and 100%; desorption levels of Cs-137 in 1N $SrCl_2$ are quite small (1-3%); the desorption levels of Cs-137 in the infinite bath scenario vary in the range of about 20 to 65%, depending on the sediment. These desorption levels (obtained subsequent to the dispersion in a 1 N $SrCl_2$) are significantly lower (a factor of 1.3 to 1.5) than the desorption levels obtained in Cs-137 single-labelled systems. Evidently, such difference is another illustration of the fixation induction of alkaline earth cations as discussed in section 2.

4. The effect of water composition on radiocaesium uptake in biota

This item was not scheduled within this program but is a major objective of our contribution in the program "Bioavailability of long-lived radionuclides in relation to their physico-chemical form in soil systems". In view of the potential importance of this issue for aquatic plants and radioecology in aquatice ecosystems, some of the manpower resources allocated to the present project were in fact devoted to this particular aspect (discussed in more detail in our interim report on soils). The essentials of the results are reported below.

A quantitative formalism was developed linking the radiocaesium TF factor (nutrient solution to plant:spinach) with overall nutrient solution composition (specified in terms of K, NH_4, Ca, Mg concentrations). Such ion exchange formalism is based on the working hypothesis that the ion exchange adsorption of radiocaesium in the "free space" is a preliminary step to the active transport process which is first-order with respect to the radiocaesium activity concentration in the exchange complex of the plant root. The quantitative relationship between the activity concentration of radiocaesium in the cell wall exchange complex and nutrient medium composition is developed on the basis of classical ion exchange theory. The formalism gives an excellent description of the radiocaesium uptake in spinach plants and is worthwhile to be tested for aquatic plants.

5. Publications

- At this stage, two doctoral dissertations (M.J.Madruga and J.Wauters) on the subject of radionuclide behaviour in sediments are in the final stage. Public defense of these theses is scheduled within 1993.

- A comprehensive list of publications on the subject of radiocaesium and radiostrontium in soils and sediments (covering the various developments which have taken place in this laboratory in recent years) is now in preparation.

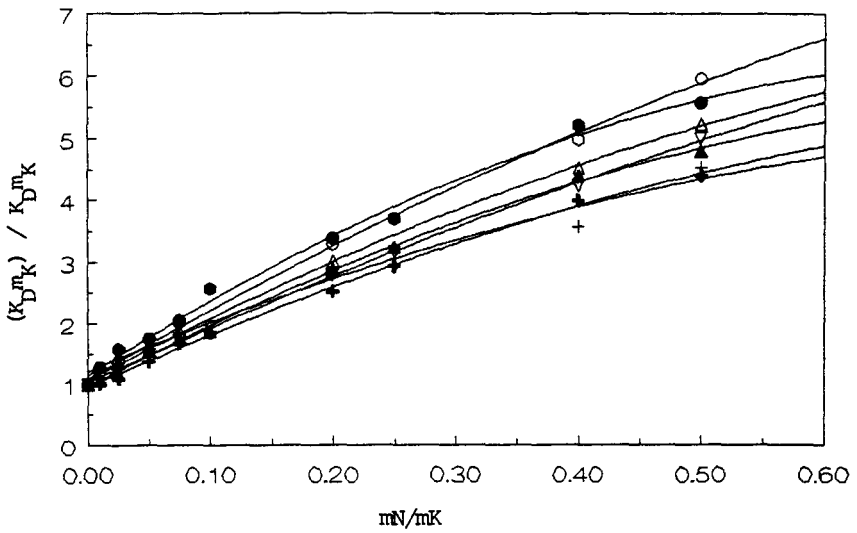


Figure 1. Effect of NH_4/K ratios on the $K_D(Cs).m_K$ product for a set of freshwater sediments.
illite (▽); freshwater pond (+);Ravenglass (△);
Tejo river (○); Rhône (✤); Moselle (▲); Loire (●).

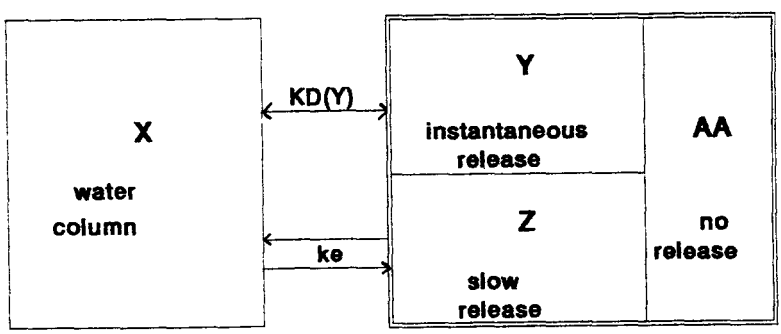


Figure 2. Three-box model for describing ^{137}Cs desorption patterns from sediments: Y fraction characterized by an equilibrium constant $K_D(Y)$, Z fraction characterized by a rate constant ke.

head of project 4: Dr. FOULQUIER

II. Objectives for the reporting period.

- The Chernobyl accident showed the importance of cesium 137 in aquatic ecosystems and its fixation in the sediments. In relation with the different source terms (fallout from atmospheric weapon tests and Chernobyl, liquid wastes for nuclear plants), we will make a review of our knowledge about cesium 137 fixation in the sediment of main french rivers, Samples of these sediments will be send to the Leuven Catholic University Laboratory (professor Cremers).

- the importance of radioruthenium was shown in Chernobyl fallout and in the liquid wastes of reprocessing fuel plants. An experimental work will be done to study the fixation of ruthenium 106 in an aquatic ecosystem. During the reporting period, the transfer of this nuclide was studied from water to algae and daphnids.

III. Progress achieved including publications

1) CESIUM IN THE FRENCH RIVERS (A.Lambrechts)

France is an intereting investigation field to study the behaviour of cesium in aquatic ecosystems.

Seven french rivers are fitted with nuclear power stations: the Rhône (with 5 stations and the fuel reprocessing plant of Marcoule), the Garonne (with 1 station (Golfech), the Loire with 4 stations (Belleville, Dampierre, Saint Laurent, Chinon), the Seine with 1 station (Nogent), the Rhine with 1 station in France (Fessenheim) but under the influence of other stations upstream (Gosgen, Beznau, Liebstat, Muhleberg), the Moselle with Cattenom, the Meuse with Chooz.

In the final report we'll give detailed granulometric composition of the sediments. Roughly, differences exist between the rivers, some like Rhone, Meuse, Rhine have silty sediment, others like Moselle, Garonne, Loire have sandy sediments.

The 137 cesium concentration was studied upstream and downstream the nuclear plants. The concentrations of this nuclide in the different rivers sediments from 1989 to 1993 was:

upper Rhône (upstream from Creys)	18 Bq/kg DW
middle Rhône (from Creys to Marcoule)	30 Bq/kg DW
downer Rhône (downstream from Marcoule)	160 Bq/kg DW
Loire (Belleville to Chinon and downstream from Chinon)	13-23 Bq/kg DW
Garonne (upstream and downstream from Golfech	4-3 Bq/kg DW
Seine	13-15 Bq/kg DW
Meuse (upstream and downstream from Chooz)	31-80 Bq/kg DW
Moselle (uptream and downstream from Cattenom)	6-47 Bq/kg DW
Rhin (upstream and downstream from Fessenheim)	65-56 Bq/kg DW

During the next period, calculations will try to show correlations between the size and the mineralogy of particles and the sediment concentrations in cesium.

2) RUTHENIUM 106 EXPERIMENTAL TRANSFER MODEL (F.Vray & J.P.Baudin)

The experimental model is constituted with several components:

- Rhône water,
- Rhône sediment both sampled upstream from Cruas,
- Primary productors, which are two species of algae (*Scenedesmus,Chlorella*) these two species present differences in morphology, size, and digestibility by their consumers.

- First order consumers (*Daphnia magna,* which is a planctonic species)
- Second order consumers *(Gambusia)*
- Third order consumer (trout).

Three transfer experiments were achieved in the reporting period:
- from water to algae
- from water to daphnids
- from algae to daphnids.

EXPERIMENTAL TRANSFER OF RUTHENIUM FROM WATER TO ALGAE

The contamination of the two species was done at several ages of the population. The fixation is higher for *Scenedesmus* than for *Chlorella.* The concentration factor is 1000 for *Scenedesmus* and 600 for *Chlorella*. For the two species, elimination is caracterised by a 0,2 day rapid biological half-life and 15 days for slow one.

EXPERIMENTAL TRANSFER OF RUTHENIUM FROM WATER TO DAPHNID.

Uptake and depuration were realised in two different aquariums where experimental conditions were similar. Figure 1 shows accumulation and depuration curves of daphnids when the water concentration was 100 and 500 Bq/ml.

Accumulation and depuration curves are similar for the 2 water concentration. Accumulation shows a concentration factor of 130 at the steady state (11 days). Elimination is caracterised by two bilogical half-lives: 6 minutes and 30 hours.

EXPERIMENTAL TRANSFER OF RUTHENIUM FROM ALGAE TO DAPHNIDS

Algae were raised in batch with 3 liters of Bristol solution enriched with natrium and carbon dioxyde, under sterile conditions, at 20°C, 16 hours of light and 8 hours of night. After 14 days of culture, algae were contaminated with 500 Bq per ml of ruthenium 106. 3 to 5 days after, algal cells were filtered and given to daphnids as one meal a day. Before feeding, daphnids were rinsed, aquarium walls were cleaned, and fresh filtered Rhône water was put in the aquariums.

The radioactivity of food was estimated by comparison of filtered and unfiltered culture medium and numeration of algae.

The parameters computed were:

- the trophic transfer factor (TTF) which measures the bioaccumulation of ruthenium during accumulation phase (concentration in daphnia/concentration in algae),
- and retention factor (R) in depuration phase (C_t/C_0).

Figure 2 shows the transfer of ruthenium to daphnids via *Scenedesmus and Chlorella.*

The elimination curve was similar to that obtained during the direct transfer (Figure 3). Depuration has 2 biological half-lives:
- 5 minutes and 2 days for *Scenedesmus*,
- 35 minutes and 30 hours for *Chlorella.*

The relative importance of the transfer of ruthenium by food (in a medium where ruthenium concentration is constant and a phytoplanctonic bloom occurs) is after 40 days (time life of daphnids) 70% via scenedesmus or 15% via chlorella.

In conclusion, the trophic transfer factor is not correlated with the digestibility of algae but with their morphology. The depuration process in daphnia are very fast. Ruthenium is not metabolised. As their contamination is high (FC = 130) daphnids will transfer ruthenium to their predators, specially when phytoplancton bloom occurs.

Publication on these transfers is in progress.

Fig.1 Transfer of 106 Ru from water to daphnids

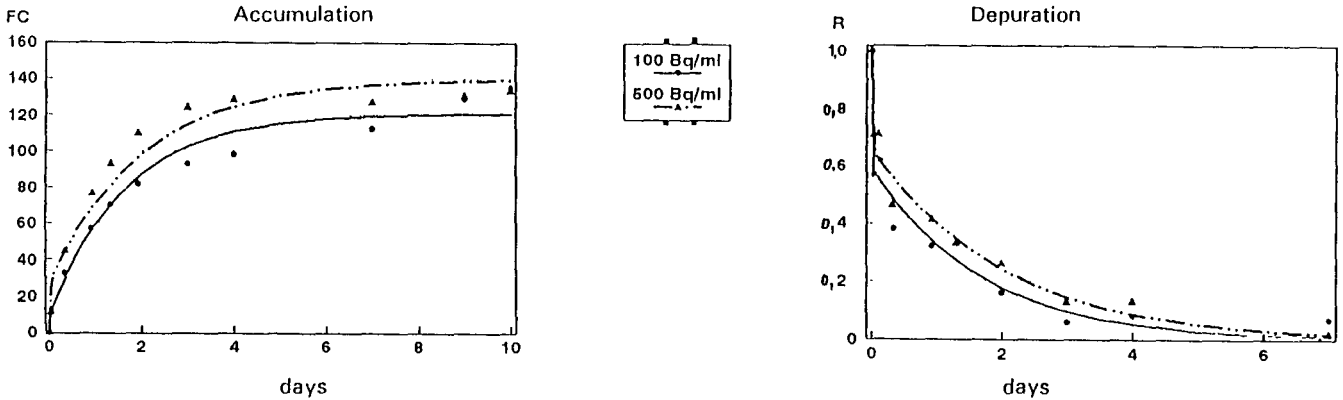


Fig.2 Transfer of 106 Ru from algae to daphnids

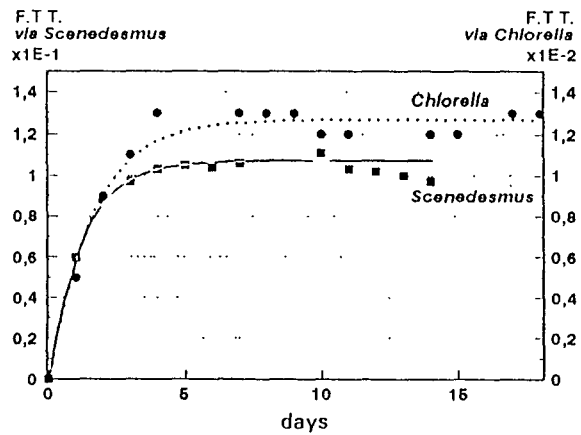
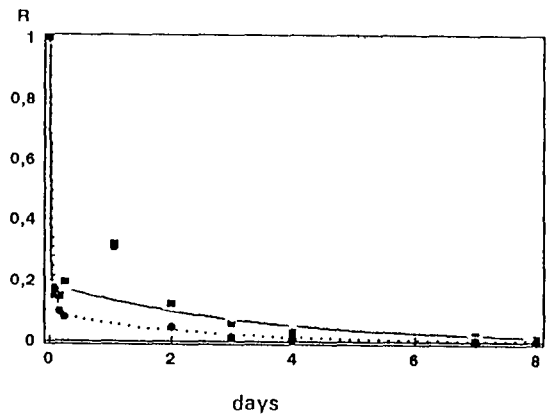


Fig.3 Depuration of daphnids



Head of project 6: Dr. Umberto Sansone, ENEA-DISP, Italy

II. Objectives for the reporting period

Role of organic and inorganic composition of suspended materials on caesium sorption-desorption processes.

Description of the experimental activities.

III. Progress achieved including publications

The aim of this research is to study the radiocaesium transfer processes, between dissolved and particulate phases, in 2 rivers located in the north-eastern part of Italy (Stella and Tagliamento rivers).

The Stella river flows into the Marano and Grado Lagoons, while Tagliamento river flows directly into the sea (Adriatic sea), west of the Marano and Grado Lagoons, and collects most of the water from the western alpine highlands. The terminal part of these rivers is subject to tidal upflow from the sea that reaches its maximum inside the rivers in dry periods.

Materials and Methods

Water and the different fractions of suspended particles were collected seasonally along the above mentioned rivers, using devices capable of performing size fractionation of suspended solids and with a set of columns containing ion-exchange resins to fix Cs-137 dissolved in the water.

The ENEA-DISP sampling system is designed to gather different fractions of suspended particles, using 4 different cylinder cartridges (from 105 to 0.45 um). 180 samples of water and suspended materials were collected and analyses are in progress.

The amount of suspended materials were determined drying and weghing the filters before and after the filtration of water.

To fix caesium dissolved in water and to determine if a saturation was reached in the resins during each sampling, two columns of NCFC resin connected in series were used (50 ccand 25 cc).

The efficiency of the filters were checked using a Coulter Multisizer (Counter Electronics Ltd.).

Cs-137 concentrations will be determined in all the samples by gamma spectrometry using HPGe detectors with 30% efficiency at 1332 keV (Co-60). All samples were counted the time necessary to achieve a counting standard deviation less than 5%.

C and N content will be determined in suspended particles samples using a CHN Elemental Analyzer.

The mineral constitution (X-ray diffraction) of suspended particles will be also determined.

In order to evaluate the effects of parameters influencing the solid-liquid partitioning of radiocaesium and stable elements, Kd values will be determined for the different fractions of the suspended solids, sampled at locations of different salinities.

Head of Project 7: Prof.Dr.O.Vanderborght
Dr.R.Blust

II. Objectives for the reporting period:

Characterisation of the interactions of radionuclides with fish gill to construct a model of the transport of radionuclides through the gill solution-body interface. Effect of short-term and long-term effects of different calcium levels in the water on the uptake of radio-cobalt by the carp, Cyprinus carpio.

Objectives for the next period:

Study of the transport kinetics of radio-cobalt and radio-cesium across fish gills and the effects of different modulators of facilitated and active transport processes on the transport process. Integration of the model developed for the speciation of the radionuclides in the environment and the model that is being developed for transport across gills.

Progress achieved during the reporting period:

The effect of external calcium upon the uptake of radio cobalt by the gills of the carp, Cyprinus carpio, was studied at various environmental calcium levels. This because 1) the acclimation of fish to various external calcium concentrations effects the number of chloride cells, and so also the number of calcium binding sites. 2) Environmental calcium levels change the ion and water permeability of membranes and the transmembrane potential of the gills. All these processes may influence the transport of metalsacross the gill epithelium.
In the experiments we used ^{57}Co, ^{60}Co and ^{45}Ca as tracers for both cobalt and calcium uptake by the common carp, Cyprinus carpio. We used this freshwater fish species since they have a wide geographic distribution, they are generally accepted as test organisms (OECD, EPA and EC) and they can easily be acclimated and exposed to a wide range of various environmental conditions. In this study we used fish which were acclimated to a variety of environmental calcium concentrations. In the cobalt uptake experiments fish of each acclimation group were transferred to artificial fresh water with the same total range of calcium levels as used during the acclimation period. This enables us to study the effect of acclimation as well as the effect of an acute

of each acclimation group were transferred to artificial fresh water with the same total range of calcium levels as used during the acclimation period. This enables us to study the effect of acclimation as well as the effect of an acute exposure to a certain external calcium level on the uptake of cobalt by the fish.

The results show that if the environmental calcium level is raised, the uptake of cobalt by the fish clearly decreases. The uptake of cobalt from the water is highly dependent on the environmental calcium concentration of both acclimation and test water. However, an increase of the calcium concentration of the experimental water, used during the exposure of the fish to the radiotracers, results in a much more profound decrease of cobalt uptake than does the elevation of the calcium level in the water used during the acclimation period.
Increasing the concentration of calcium in the water increases the ionic strength of the solution. This results in a decrease of the activity of the free cobalt species with 40% within the range of the used experimental calcium concentrations. However the decrease in free cobalt activity can only account for approximately 30 % of the decrease in cobalt uptake.
Using a mathematical model, which takes into account the Ca^{2+} activity of the acclimation water and both Co^{2+} and Ca^{2+} activities of the experimental water, ($uptake=0.492*Co^{2+}*Ca^{2+}_{acclimation}{}^{(-0.051)}*Ca^{2+}_{exposure}{}^{(-0.348)}$) we can explain 88% of the variation in cobalt uptake from the water. Therefore we can conclude that the activity of calcium in both acclimation and experimental water significantly effects the uptake of cobalt.

Subsequent experiments were performed to study the nature of the interaction between the external calcium concentration and the uptake of cobalt. Fish were acclimated to a fixed calcium concentration, after which uptake experiments were performed in media with one fixed calcium concentration and various cobalt levels. We found that the uptake of cobalt increases with increasing environmental concentrations of the transition metal. This even at an environmental calcium concentration which inhibited the metal uptake in the first set of experiments. The increase in cobalt uptake, in the studied metal concentration range of 10^{-3} to 10^{3} µM Co^{2+}, was consistent with a Michaëlis-Menten like behaving system. These results, and the fact that the uptake of the metal decreases with increasing environmental calcium concentration, could indicate that cobalt uptake from the water is competitively inhibited by the external calcium. However, we have not yet

determined the kinetic parameters necessary to confirm this hypothesis.

Publications for the reporting period:

Blust, R. and Vanderborght, O. Radioecology in the freshwater environment. Models of transfer and uptake. In: Introduction to Radioecology, (book in preparation).

Comhaire, S, Blust, R., Van Ginneken, L., D'Haeseleer, F, Vanderborght, O. The effect of calcium on the uptake of cobalt by the gills of the carp, Cyprinus carpio. Journal of Experimental Biology (submitted).

Head of project 8: Dr. Fernández

II. Objectives for the reporting period

The aim for this period was to provide, under the current knowledge of plant membrane transport (Maathuis and Sanders, 1992) a simple model for ^{134}Cs accumulation in *Riccia fluitans*. Because of the similarities with K^+, it was assumed that: radio cesium is incorporated into the cells through potassium channels, being the driving force for the uptake the electrochemical gradient for cesium ($\Delta\mu_{Cs}/F$). This value is proportional to the difference between the membrane potential (Em) and the Nernst potential for Cs (E_{Cs}^N),
$\Delta\mu_{Cs}/F = z\ (Em - E_{Cs}^N)$, where z is the charge for Cs, and F is the Faraday constant.

III. Progress achieved including publications.

Methods.
Experiments were performed in the aquatic liverwort *Riccia fluitans*. ^{134}Cs uptake was measured following the accumulation in the plant in a standard artificial pond water (0.1 mM KCl, 0.1 mM NaCl, 0.1 mM $CaCl_2$ buffered with MES-TRIS pH 7.3). Concentration factor (CF) was measured when no appreciable change in the external radio nuclide concentration was observed.
Since CF is the ratio between the internal and the external concentration, the Nernst potential for Cs was computed from the expression,
$E_{Cs}^N = RT/zF \ln CF^{-1}$
where R is the gas constant, and T is the absolute temperature.
Em was measured, by means of classical electrophysiology, in parallel experiments performed under the same conditions.

Results
CF for this species in the standard APW, was 130.0±3.4 (n=9). This figure give a Nernst potential for ^{134}Cs of -123 mV at 20°.
The addition of tetramethylammonium chloride (TEA), a well known potassium channel blocker (Bentrup, 1990), caused a decrease in CF with respect to control of 43%.
CF values decreased dramatically in the presence of different external potassium concentrations (Fig.1), being minimum at the highest external potassium concentration. The addition of 0.1 mM CN^- caused a further decrease in CF for every external concentration of K^+ (Fig.1). Concentration factor for K^+ follow, in short term experiments, the same pattern than CF for ^{134}Cs, but under any treatment, the figures of CF for K^+ were higher than those obtained for ^{134}Cs.
For a given external K^+ concentration, CF exhibited a saturation kinetic with respect to the electrochemical gradient for ^{134}Cs. Fig. 2 shows an example at an external K^+ concentration of 0.1 mM.
CF in *R. fluitans* expressed on a logarithmic basis, is proportional to diffusion potential (E_D, the membrane potential measured in the

presence of a metabolic inhibitor CN^- for example). Over the range of external K^+ concentration assayed, this line is parallel to the relationship between CF, expressed on a logarithmic scale and Nernst potential for ^{134}Cs (Fig.3).
Recently, special systems for K^+ uptake have been described for K^+ starved plants. In the fresh water alga *Nitella translucens* Walker and Sanders (1991), demonstrated the existence of a Na^+ coupled K^+ uptake. In K^+ starved *R. fluitans*, the addition of K^+ caused a depolarization of Em. Such result point out a special high affinity system for K^+ uptake, but in contrast with the results of Walker and Sanders (1991), this transport is not Na^+ dependent. This system exhibit a similar affinity for Cs^+, but the maximum rate of transport is only the 36% with respect to K^+ (Fig. 4). In this species the system does not transport Na^+.
The strong inhibition of Cs^+ accumulation caused by K^+, and the results obtained with classical potassium channel blockers (previously reported) support the assumption that Cs^+ enters the cells trough K^+ channels. In K^+ starved plants, a high affinity transport system is induced. Cesium binds to such system with the same affinity than potassium, but this ion is transported much more rapidly than Cs^+. The system is independent on the presence of Na^+ in the external medium, so it is possible than K^+ (eventually Cs^+) be incorporated into cells by means a proton cotransport.

Objectives for the next period.

1.- pH dependence of ^{134}Cs uptake and accumulation.
2.- Influence of potassium channel blockers (different to TEA) on ^{134}Cs transport and accumulation.
3.- Characterization of the high affinity transport system for K^+ and Cs.
4.- Influence of K^+ (Cs^+) uptake on cytoplasmic pH in K^+ starved plants.
5.- Study of the effect of growth rate on CF for ^{134}Cs in green freshwater algae.

For the achievement of the objectives 1 to 4, we will use *Riccia fluitans*. The experiments will be done by using classical radio nuclide uptake experiments, classical electrophysiology, and ion selective micro electrodes.
The objective 5 is tentative. An unicellular green algae will be used, changing the growth rate by changing the dilution rate in chemostats.

Publications.

Fernández J.A., Vaz Carreiro M.C., Gil Corisco J.A., Niell F.X. and Diez de los Rios A. (in revision). Mechanisms of ^{134}Cs accumulation in *Riccia fluitans*.

Sanders D., Corzo, A., and Fernández J.A. (in preparation). High affinity K^+ transport in K^+ starved *Riccia fluitans* plants.

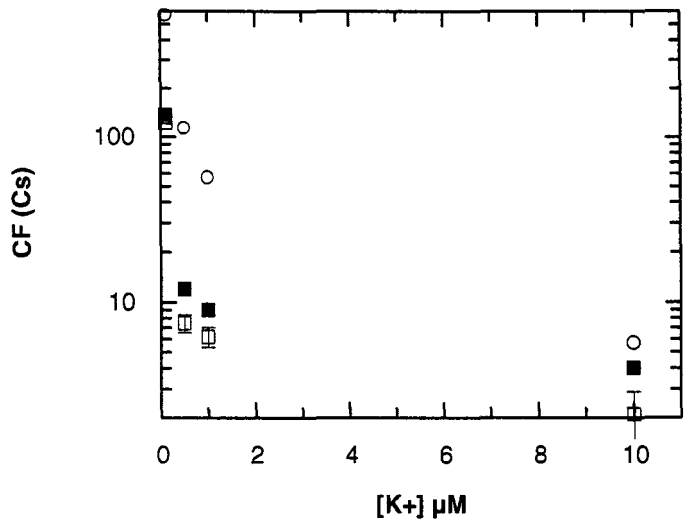


Figure 1.- Variation of concentration factor for ^{134}Cs in the absence (closed squares) and in the presence (open squares) of 0.1 mM NaCN, and K^+ (open circles) in *Riccia fluitans*.

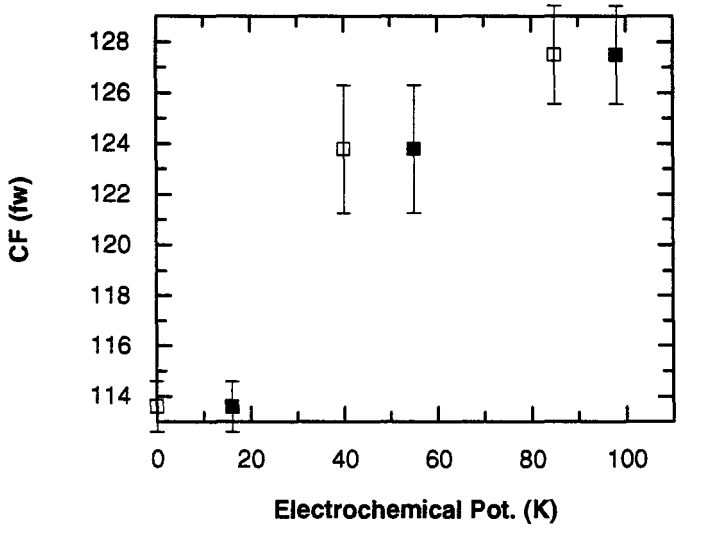


Figure 2.- Variation of concentration factor (CF), expressed on a fresh weight basis, for *Riccia fluitans*, as a function of the electrochemical potential (expressed as positive numbers) for potassium (open squares) and cesium (closed squares).

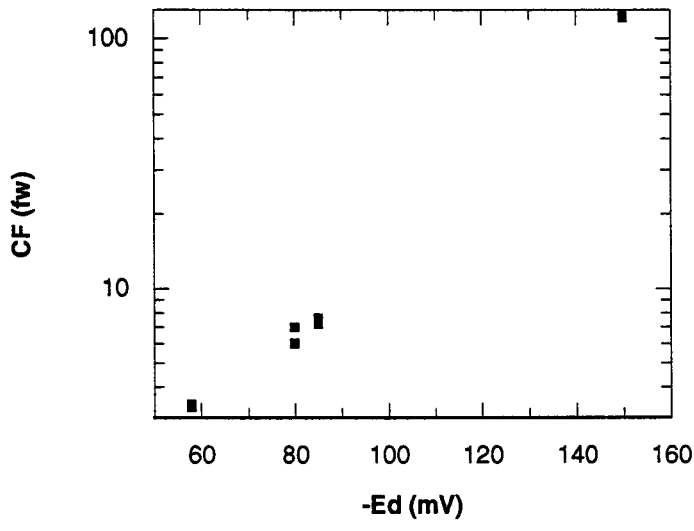


Figure 3.- Variation of concentration factor, expressed on a fresh weight basis (CF) as a function of diffusion potential (Ed) in *Riccia fluitans*.

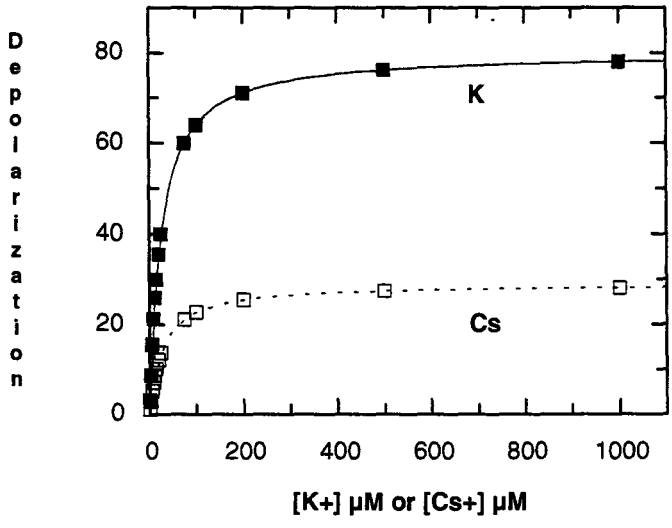


Figure 4.- Depolarization of membrane potential in K^+ starved *Riccia fluitans* plants after the addition of kown amounts of K^+ or Cs^+. Kinetic parameters are Vm (K^+)= 80 mV, Km (K^+)= 25 µM and Vm (Cs^+)= 29 mV, Km (Cs^+)= 28 µM.

Head of project 10: Dr. R.N.J. Comans

II. Objectives for the reporting period

1) To measure *in-situ* radiocaesium K_D's in freshwater sediments differing in concentrations of illitic frayed edge sites and levels of competing cations (NH_4^+, K^+) and to identify the relationships between *in-situ* K_D's and fundamental sediment/pore water characteristics
2) To develop a procedure which allows for reliable measurement of the exchangeability of Chernobyl- and bomb-radiocaesium
3) To study the kinetics of radiocaesium release (desorption) from contaminated sediments

III. Progress achieved including publications

Background.

In order to allow transport models to make reliable predictions of radiocaesium mobility, the K_D-value for this radionuclide needs to be related to fundamental sediment and pore water characteristics and to the contact time between the radionuclide and the solid phase. In the reporting period, progress has been made which has substantially increased the possibilities to fulfil these needs:

1. Compilation of all *in-situ* K_D-values, measured in the framwork of this project to date, in a $\log K_D$-$\log NH_4^+$ plot, has revealed that the data follow a straight line with a slope of -1. This releationship is consistent with ion-exchange theory, with pore-water NH_4^+ as the competing ion controlling the radiocaesium distribution coefficient. The vertical variation in the plot (the offset) is about one order of magnitude or less in K_D and suggests, on the basis of ion-echange theory, that the product of the selectivity coefficient and the concentration of the caesium selective (frayed edge) sites in all of these European sediments would also vary by less than a factor of 10. These predictions agree well with the experimental work of the group of Prof. Cremers (KUL) on the same sediment

samples. Both the earlier (1991) and recent (1992) results from Esthwaite are consistent with the above relationship.

2. A procedure is being developed in which Chernobyl- and bomb-radiocaesium is extracted from sediments with multiple 0.1 M NH_4^+ extractions, followed by preconcentration of radiocaesium from the extracts and low-background measurement. Prior removal of the NH_4^+ from the extracts appeared necessary in order to obtain recoveries of about 90%. The first results of this procedure are promising and indicate the exchangeability of Chernobyl-radiocaesium in Ketelmeer sediments from 1987 to vary between 1 and 7%.

3. Long-term desorption experiments have been started to measure the extent and kinetics of Chernobyl- and bomb-radiocaesium release from sediments.

Progress Report

Contract: **F13P-CT920046** **Sector: A21**

Title: Mechanisms governing the behaviour and transport of transuranics (analogues) and other radionuclides in marine ecosystems.

1)	Mitchell	Univ. Dublin - College
2)	Gascó	CIEMAT
3)	Guéguéniat	CEA - Cadarache
4)	Papucci	ENEA
5)	Woodhead	MAFF
7)	Sánchez-Cabeza	Univ. Barcelona - Autónoma

I. Summary of Project Global Objectives and Achievements

The global objective of this programme is to define the fundamental parameters governing the transport and behaviour of transuranics and other radionuclides in diverse marine environments and provide data of a universal character for use in real, predictive models based on fundamental mechanisms. Specific objectives successfully accomplished in the course of the reporting period included: (a) characterisation of radionuclides in the Sellafield source term with emphasis on the extent and the size fractionation of the colloidal components, and identification of possible transformations of forms upon discharge into the Irish Sea (MAFF); (b) examination of the physico-chemical speciation and colloidal association of transuranics in the near- and the far-field throughout the Irish Sea (MAFF and UCD); (c) comparison of the efficacy of alternative and/or complementary techniques for the identification of the colloidal transuranic fraction (UCD); (d) identification of the pathways for the re-distribution of plutonium associated with sediments in mega-tidal areas of the English Channel, and elucidation of the behaviour of dissolved and particulate Pu in the vicinity of the La Hague plant and the Seine Estuary (CEA); (e) characterisation of the physico-chemical speciation of plutonium and americium, including colloidal fractions, in the constituent water masses of the western Mediterranean (UCD, UAB and ENEA); (f) determination of vertical profiles of ^{137}Cs in the water column and in sediments in the western Mediterranean, in order to establish the ^{137}Cs inventories in both compartments and evaluate the role of different water masses in the horizontal and vertical transport of long-lived radionuclides in the basin (ENEA); (g) measurement of transuranic concentrations in the particulate and filtered fractions along an east-west transect between Sardinia and the Gulf of Vera (UCD); (h) establishment of the submarine topography off Palomares (CIEMAT and ENEA); (i) evaluation of the inventories of plutonium, americium and gamma-emitting nuclides in sediment cores in order to map the distribution of these radionuclides in the Palomares zone and identify the source terms (CIEMAT and UAB); (j) assessment of the role of zooplankton as an agent for the remobilisation of plutonium (and other radionuclides) and, in particular, the experimental determination of representative concentration factors for zooplankton in western Mediterranean waters (UAB). In addition to the above achievements, the Group can report the development and application of various technical and analytical innovations in the course of the programme, and a substantial exchange of expertise amongst the partners. A number of collaborative marine research expeditions has also been undertaken in the marine zones referred to above.

Head of project 1: Dr. Mitchell

Objectives for the reporting period

(1) Examine the physico-chemical speciation of plutonium at (a) a deep water station (vertical profile) in the open waters of the western Mediterranean and (b) in coastal waters near Palomares (Spain), and compare the results with those obtained previously in the Irish Sea by our laboratory;
(2) Determine the fractions of plutonium and americium in a colloidal form in filtered sea water (<0.45 μm) at three stations in the western Mediterranean (Gulf of Vera) using the technique of differential sorption on aluminium oxide;
(3) Measure plutonium and americium concentrations in the particulate and filtered fractions in surface sea water sampled at five stations along an east-west transect between Southern Sardinia and the Gulf of Vera; and
(4) Quantify the fractions of plutonium and americium in colloidal form in filtered water throughout the Irish Sea and, in particular, apply a new technique, based on a combination of ultrafiltration and differential sorption, to the determination of the physico-chemical speciation of plutonium in different size-fractions (ultrafiltrate) of sea water sampled near the Sellafield outlet.

Objectives for the next reporting period

(1) Examine the oxidation state distribution of plutonium at a deep water station (vertical profile) in the Catalan-Balearic Sea with emphasis on the upper part of the water column;
(2) Examine the physico-chemical speciation of plutonium and its variation in the so-called dissolved phase in a large surface water sample from the Catalan-Balearic Sea upon ultrafiltration through polysulphone membranes with nominal molecular weight cut-offs (NMWL) of 100 kD, 10 kD, 3 kD and 1 kD, respectively;
(3) Compare the fractions of plutonium and americium in chemically reduced and colloidal forms in large (1000 l) near-surface water samples collected in the vicinity of the Barcelona offshore zone and in the open waters of the western Mediterranean;
(4) Repeat (4) above in order to corroborate certain fundamental deductions arising from this complex and extensive experiment; and
(5) Complete the development of a technique based on continuous extraction using suitable recyclable organic solvents, in order to measure the fractions of plutonium and americium (and other radionuclides) in the form of neutral organic complexes in sea water.

Progress achieved including publications

Sampling and analysis

Four x 270 l water samples were collected, using *Gerhard-Ewing* samplers, at Station 3 (37° 49´N, 02° 32´E) in the western Mediterranean, where a significant Levantine water signal was anticipated. A further set of three x 270 l samples were collected in the Gulf of Vera, close to Palomares. These, and all other large water samples taken, were filtered through *Millipore* membrane screen filters (0.45 μm) prior to further processing. The oxidation state groups of plutonium in the filtered fractions were then separated from one another using a scaled-up version of the selective co-precipitation (NdF_3) method of Lovett and Nelson

(1981). In essence, two isotopic tracers, ^{242}Pu(IV) and ^{236}Pu(V), in chemically reduced and oxidised forms respectively, are used to monitor the separation of the two oxidation state groups of ^{239,240}Pu and to establish the radiochemical yield of each. In every case, separations were carried out on board as soon as possible after sampling, in order to minimise changes in oxidation state distribution, inevitable when storage times are long.

Three x 1000 l samples of filtered sea water collected in the Gulf of Vera were passed through sets of aluminium oxide beds (4), in order to determine the fractions of plutonium and americium in colloidal form in surface waters. Each bed was subsequently analysed for plutonium and americium at UCD, using well-tested radioanalytical techniques.

Five x 250 l surface sea water samples were collected along an east-west transect between southern Sardinia and the Gulf of Vera. These were filtered and pre-concentrated for subsequent plutonium and americium analysis by co-precipitation with ferric hydroxide.

Filtered sea water (< 0.45 μm) from the Sellafield offshore zone was passed through a selection of *Filtron*-supplied tangential-flow ultrafiltration cassettes mounted in *Millipore*-supplied *Pellicon* assembly units and operated in concentration (recyclable) mode. The permeates obtained separately using 10 kD, 3 kD and 1 kD cassettes, respectively, were then treated using the dual tracer coprecipitation technique referred to above, in order to establish the oxidation state distribution of plutonium in each size fraction. Separate samples of permeate from each size fraction were passed through aluminium oxide sorption bed sets in order to quantify the colloidal Pu fraction. Our objectives were (a) to compare the results from differential sorption analysis and tangential flow ultrafiltration analysis in the estimation of the size of the colloidal Pu fraction, (b) to establish an upper limit to the physical size of the particles involved and, (c) to examine the relationship between reduced Pu and colloidal Pu. Finally, total ^{239,240}Pu and ^{238}Pu concentrations in the retentates from the different size fractions were determined by means of the $Fe(OH)_3$ co-precipitation technique. Complete details of the methodology employed above can be found elsewhere (Vives i Batlle, 1993)

Results

Physico-chemical speciation of plutonium and americium in the western Mediterranean Sea and the Gulf of Vera

Plutonium and americium concentrations on suspended particulate and reduced and oxidised plutonium concentrations in filtered sea water sampled at various depths in both the western Mediterranean Sea and the Gulf of Vera are given in Table 1, while the percentages of plutonium and americium on suspended particulate and the percentage of plutonium(V,VI) in filtered water, together with the matching DOC concentration from each of the stations analysed, are summarised in Table 2.

The vertical distribution of plutonium in the open waters of the western Mediterranean Sea is quite different from that observed in the shallow waters of the Irish Sea. Clear evidence of a sub-surface maximum at about 500 m is evident from the data.

The mean ^{238}Pu/^{239,240}Pu activity ratio in filtered water, namely 0.043 ± 0.019 ($n = 10$), is consistent with values for Mediterranean waters reported previously by other workers and is only slightly higher than that found in the open ocean waters of the Atlantic and the Pacific (attributable to global fallout).

Table 1. Plutonium and americium concentrations on suspended particulate matter and plutonium speciation in filtered (< 0.45 μm) sea water sampled in the western Mediterranean Sea and the Gulf of Vera (July-August, 1991)

Location (Depth)	Fraction	Concentration (mBq m^{-3}) ^{238}Pu	$^{239,240}Pu$	^{241}Am	$^{238}Pu/^{239,240}Pu$	$^{241}Am/^{239,240}Pu$
Gulf of Vera:						
37°10´N	S. Load	ND	1.08 ± 0.24	0.65 ± 0.09	-	0.60 ± 0.16
01°40´W	Reduced	ND	1.25 ± 0.14	-	-	-
(773 m)	Oxidised	0.64 ± 0.09	25.7 ± 1.2	-	0.025 ± 0.004	-
37°10´N	S.Load	ND	1.21 ± 0.22	0.42 ± 0.09	-	0.35 ± 0.10
01°40´W	Reduced	0.40 ± 0.09	8.4 ± 0.5	-	0.047 ± 0.011	-
(3 m)	Oxidised	0.28 ± 0.06	9.4 ± 0.4	-	0.030 ± 0.006	-
37°11´N	S. Load	0.02 ± 0.03	0.59 ± 0.07	ND	0.04 ± 0.06	-
01°48´W	Reduced	0.14 ± 0.03	4.4 ± 0.3	-	0.032 ± 0.007	-
(3 m)	Oxidised	0.56 ± 0.07	7.6 ± 0.4	-	0.074 ± 0.009	-
Western Mediterranean (Stn. 3):						
37°49´N	S. Load	ND	0.76 ± 0.16	0.77 ± 0.11	-	1.0 ± 0.3
02°32´E	Reduced	ND	1.30 ± 0.15	-	-	-
(2745 m)	Oxidised	0.68 ± 0.11	23.5 ± 1.2	-	0.029 ± 0.005	-
37°49´N	S. Load	ND	0.31 ± 0.05	0.82 ± 0.13	-	2.7 ± 0.6
02°32´E	Reduced	ND	1.67 ± 0.14	-	-	-
(1200 m)	Oxidised	2.5 ± 0.6	19.6 ± 1.8	-	0.12 ± 0.03	-
37°49´N	S. Load	ND	0.70 ± 0.10	ND	-	-
02°32´E	Reduced	ND	3.77 ± 0.24	-	-	-
(515 m)	Oxidised	1.32 ± 0.19	25.3 ± 0.8	-	0.052 ± 0.008	-
37°49´N	S. Load	ND	0.94 ± 0.13	ND	-	-
02°32´E	Reduced	0.09 ± 0.02	3.67 ± 0.22	-	0.025 ± 0.006	-
(15 m)	Oxidised	0.65 ± 0.06	8.9 ± 0.4	-	0.073 ± 0.006	-
Mean	-	-	-	-	0.043 ± 0.019 (n = 10)	1.2 ± 1.1 (n = 4)

Coordinates correspond (in descending order) to *N/O Bannock* Stations 10, 13 and 3, respectively

Table 2. Percentage of plutonium on suspended particulate and percentage of plutonium(V,VI) in filtered (< 0.45 μm) sea water sampled throughout the western Mediterranean Sea

Location Depth	% ^{239,240}Pu in particulate	% ^{239,240}Pu(V,VI) in filtrate	DOC (mg/l)
Gulf of Vera (Stn. 10):			
773 m	3.9 ± 0.8	95.4 ± 0.5	6.3 ± 0.1
3 m	6.4 ± 1.1	52.7 ± 1.7	4.7 ± 0.1
Gulf of Vera (Stn. 13):			
3 m	4.7 ± 0.6	63.4 ± 1.8	5.4 ± 0.1
Western Mediterranean (Stn. 3):			
2745 m	3.0 ± 0.6	94.8 ± 0.6	4.5 ± 0.1
1200 m	1.4 ± 0.3	92.2 ± 0.9	4.0 ± 0.1
515 m	2.4 ± 0.3	87.0 ± 0.8	3.5 ± 0.1
15 m	7.0 ± 0.9	70.7 ± 1.6	3.8 ± 0.1

The percentage of plutonium in a reduced chemical form in the western Mediterranean water column (Station 3) was found to increase from 5% at a depth of 2745 metres to almost 30% at a depth of 15 metres, most if not all of this increase taking place in the top 500 metres.

It is interesting to compare the vertical distribution of the oxidation states of plutonium in the Mediterranean Sea with that in open ocean waters. Analyses of the chemical speciation of plutonium in Atlantic waters carried out by Cochran *et al.* (1987) indicate that ≥ 85% of plutonium in this environment was in an oxidised form at all depths, in clear contrast to the Pacific Ocean (Nelson *et al.*, 1984), where the percentage of plutonium(V,VI) was much lower, except near the bottom. With the exception of the near-surface water, in which the proportion of reduced plutonium is significant at 30%, our data is consistent with those reported for the Atlantic, but differs noticeably from those for Pacific waters. Differences in the chemical speciation of plutonium observed in these environments have been attributed to differences in the nature of the source term.

The percentages of reduced plutonium in (filtered) surface waters at Station 10 and Station 13 in the vicinity of the Palomares offshore zone were 47% and 37%, respectively. Thus, the proportion of plutonium(III,IV) in the surface waters of the Vera Gulf was significantly higher than in the open waters of the western Mediterranean (Station 3). Factors which can account for the higher percentage of plutonium(III,IV) in coastal zones include (a) the presence of higher pollutant and/or DOC concentrations in coastal regions and (b) the existence of significant water run-off in the proximity of the coast. In fact, we found that DOC concentrations in surface sea water were higher in the vicinity of the Gulf of Vera than in open waters, an observation which may, in part, account for the significant increase in the percentage of plutonium(III,IV) as one approaches the coastline. Moreover, our data proved

to be in good accord with the results of laboratory studies on the influence of DOC concentrations on the chemical speciation of plutonium in natural waters.

In contrast, the percentage of plutonium(III,IV) in near-bottom waters in the Gulf of Vera (Station 10) was found to be marginally less than 5%. The latter result is in good accord with our data from the vertical profile at Station 3 and constitutes a further confirmation that, in near-bottom waters generally, a much higher proportion of the plutonium present in the filtered fraction will be in an oxidised form as a result of the influence of enhanced biogenic activity.

For the purposes of quality control, the concentration of $^{239,240}Pu$ in surface water sampled at Station 13 was also measured using the $Fe(OH)_3$ co-precipitation technique. The result, 12.5 $\pm$ 0.5 mBq m^{-3}, was statistically indistinguishable from that determined using the NdF_3 selective co-precipitation technique, 12.0 $\pm$ 0.5 mBq m^{-3}, and is further confirmation of the efficacy of the latter methodology. More generally, our laboratory has continued its practice of regular participation in international intercomparison exercises, in addition to appropriate intercalibrations with other participants in the collaborative programme and the implementation of an extensive in-house quality assurance regime.

Representative K_d coefficients for plutonium in filtered (<0.45 m) sea water sampled in the western Mediterranean Sea were also determined in the course of this work. The mean values for total Pu and Pu(III,IV) were $(1.0 \pm 0.9)x10^5$ and $(1.1 \pm 1.5)x10^6$, respectively and are consistent with data reported previously for the eastern Irish Sea and elsewhere by this laboratory.

The percentages of plutonium and americium in a colloidal form in sea water sampled on the Continental Shelf close to Palomares were found to be in the range 5-15 % and 90-100 %, respectively. These values are similar to those observed previously by our laboratory in the open waters of the Irish Sea and, again, were determined by the technique of sequential sorption on aluminium oxide. Extensive work to assess the size fractionation of the colloidal component in Mediterranean waters using a combination of sequential sorption and ultrafiltration is presently under way and will be described in detail in the next report.

^{238}Pu, $^{239,240}Pu$ and ^{241}Am in surface sea water (Gulf of Vera - Sardinia transect)

Plutonium and americium concentrations in (filtered) surface sea water sampled along a transect from the Gulf of Vera to the island of Sardinia are given in Table 3. In general, these concentrations were lower than those recorded in earlier studies carried out in the western Mediterranean prior to the Chernobyl accident. However, our data are entirely consistent with more recent studies carried out in the Catalan-Balearic Sea and the Gulf of Lyons by UAB and IAEA-MEL. On the basis of this evidence, it is clear that transuranic levels in surface waters in the western Mediterranean zone are falling with time. This is not unexpected, given that only a very small fraction of the plutonium (and americium) originating from atmospheric nuclear weapons tests still resides in the stratosphere.

Plutonium and americium concentrations diminished significantly with distance from the coasts of Spain and Sardinia, reaching a minimum along the transect at a longitude of about 3° East. It is, therefore, clear that transuranic levels in the surface waters of the western Mediterranean are not as homogeneous as they appear to have been a decade or more ago. We believe that these variations in the lateral distribution of plutonium and americium reflect

both the reduction in direct atmospheric input and the natural advective movement and turbulent mixing of distinct water masses due to the complex oceanography of the Mediterranean.

Table 3. ^{238}Pu, $^{239,240}Pu$ and ^{241}Am concentrations in filtered sea water sampled along an east- west transect through the western Mediterranean Sea (July-August, 1991)

Location	Concentration (mBq m^{-3})			$^{238}Pu/^{239,240}Pu$	$^{241}Am/^{239,240}Pu$
	^{238}Pu	$^{239,240}Pu$	^{241}Am		
37° 11′N 01° 48′W	0.28 ± 0.05	12.5 ± 0.5	0.40 ± 0.07	0.023 ± 0.004	0.032 ± 0.006
37° 39′N 00° 26′E	0.29 ± 0.23	6.8 ± 0.5	0.37 ± 0.07	0.04 ± 0.03	0.054 ± 0.011
38° 26′N 03° 15′E	0.21 ± 0.14	7.0 ± 0.4	0.15 ± 0.04	0.030 ± 0.021	0.021 ± 0.006
38° 16′N 06° 17′E	0.42 ± 0.11	10.2 ± 0.5	0.18 ± 0.04	0.041 ± 0.011	0.018 ± 0.004
38° 36′N 08° 21′E	1.3 ± 0.3	29.7 ± 1.1	0.92 ± 0.03†	0.044 ± 0.009	-
Mean	-	-	-	0.036 ± 0.009 (n = 5)	0.031 ± 0.016 (n = 4)

†Calculated using the mean $^{241}Am/^{239,240}Pu$ activity ratio in the first four samples, namely 0.031 ± 0.014

From the isotopic composition of plutonium, i.e., the $^{238}Pu/^{239,240}Pu$ activity ratio, it is clear that the concentrations measured in this study are representative of global fallout. This is further confirmed by the fact that the mean ratio is almost identical to that reported in open oceans. In contrast, the $^{241}Am/^{239,240}Pu$ ratio in surface sea water is approximately an order of magnitude lower than the value of 0.30 ± 0.06 reported for the integrated global fallout (derived from soil measurement data) in the mid-1980's. Since the $^{241}Am/^{239,240}Pu$ activity ratio should, if anything, increase with time due to ingrowth following the decay of ^{241}Pu, it is evident that ^{241}Am is removed from the water column more rapidly than $^{239,240}Pu$. In fact, studies carried out elsewhere in 1978-79 indicate that the mean residence time of ^{241}Am in the Mediterranean Sea mixed layer (taken to be 100 m) is about four times shorter than that of $^{239,240}Pu$. This is supported by the observed increase in the $^{241}Am/^{239,240}Pu$ ratio in seabed sediments over the past decade (having allowed for ^{241}Am ingrowth).

Quantification of the colloidal fraction of plutonium and americium throughout the Irish Sea and Carlingford Lough

The aluminium oxide sorption technique has been used extensively to determine the fractions of plutonium and americium in colloidal form throughout the Irish Sea and Carlingford Lough. Our results indicate that about 50% of the plutonium and almost 100% of the americium in filtered water in Carlingford Lough is colloidal in character, whereas in the open waters of the Irish Sea the corresponding figures are 5-15% and 95-100%, respectively.

A typical Pu sorption isotherm for a sample of filtered sea water taken in the vicinity of the Sellafield offshore zone in 1992 is shown in Figure 1.

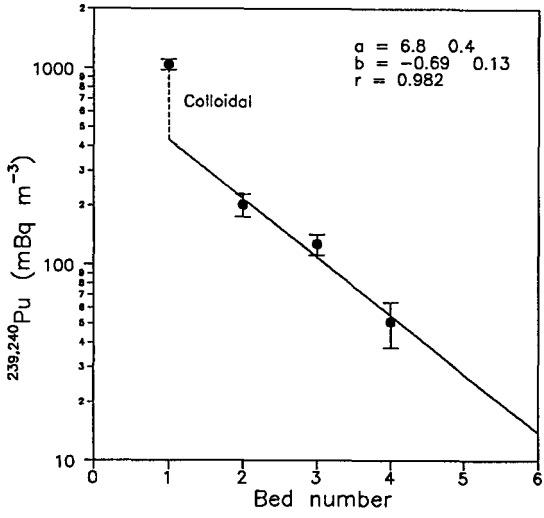


Figure 1. Pu sorption isotherm showing excess (colloidal) ^{239,240}Pu on the first bed

We observed a strong correlation between the percentage of plutonium in a colloidal form and the percentage of plutonium in a reduced chemical form, Pu(III,IV). The extent of this correlation is evidenced by the plot shown in Figure 2. In the case of americium, known to

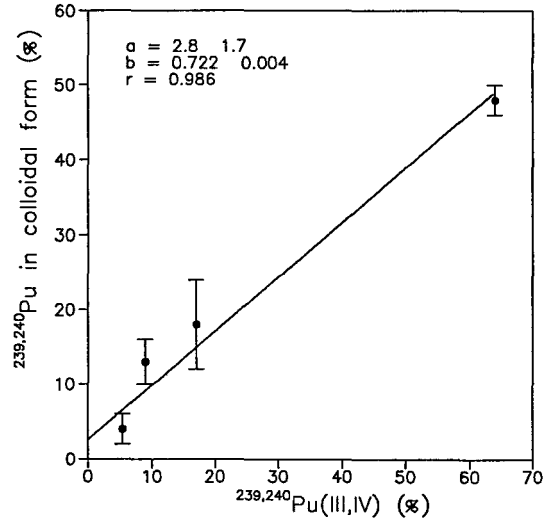


Figure 2. Correlation between colloidal Pu and Pu(III,IV) in filtered sea water in the Irish Sea

be present in sea water almost exclusively in a reduced chemical form, the correlation was even more striking, in that all of the ^{241}Am appeared to be colloidal (as experimentally defined by enhanced sorption). These are fundamental observations which have far reaching implications in regard to the transport and bio-availability of plutonium and americium and, by extension, for modelling and radiological protection.

The percentage of plutonium in reduced form (as determined by selective co-precipitation on NdF_3) has been compared with the percentage of plutonium in a colloidal form (as determined by sorption on aluminium oxide) in samples of filtrate/permeate following microfiltration/ ultrafiltration, through membranes with nominal cut-offs of 0.45 μm, 10 kD, 3 kD and 1 kD, of sea water sampled in the Sellafield offshore zone. The data indicate that a significant proportion of the plutonium present in a reduced form is retained by the 1 kD membrane, in contrast to plutonium in an oxidised form which is found to be completely soluble. Although preliminary, there is evidence which indicates that plutonium in a colloidal form is also removed upon ultrafiltration through a similarly sized membrane. Further, there is more limited evidence to suggest that the remaining plutonium, now almost exclusively Pu(V), disproportionates to Pu(VI) and Pu(IV), both of which tend to become partially hydrolysed in a relatively short period of time. Further studies to confirm these observations are presently being carried out, the results of which will be discussed in our next report.

Acknowledgments

The support and cooperation afforded the coordinator by the participants in this collaboration and by the Comission throughout the reporting period is gratefully acknowledged. UCD also wishes to acknowledge the generous facilities and technical support provided by ENEA and MAFF in the course of collaborative research expeditions in the Mediterranean and the Irish Sea in the period 1991-present. We also wish to thank our colleague J.A. Sánchez-Cabeza for his invaluable assistance during the MED'91 research expedition.

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Head of project 2: Dr. Gascó

Objectives for the reporting period

(1) To prepare and conduct an oceanographic campaign (aboard the Urania ENEA-CRN research vessel) along the Spanish Mediterranean coast and the Palomares area;
(2) To study the topography of the Palomares canyon zone;
(3) To develop an analytical method for the sequential leaching of plutonium in marine sediments;
(4) To carry out the radiochemical analyses of sediment samples collected during the Urania cruise; and
(5) To perform analytical intercomparisons for quality control purposes.

Objectives for the next reporting period

(1) To separate the ^{241}Am heterogeneities (possible *hot particles*) found in the sediments collected during the *Bannock* oceanographic campaign;
(2) To study the association of plutonium with sediments in the Palomares zone;
(3) To examine the terrigenous input of other zones in the Spanish Mediterranean;
(4) To evaluate the more relevant results in relation to the two most probable mechanisms of plutonium transport in this zone namely, (a) transport over a long timescale through the canyons (influence of the topography and marine dynamics), and (b) transport due to its chemical form/association.

Progress achieved including publications

(1) A sediment sampling campaign has been carried out (aboard the *Urania* research vessel) in the zone of the Palomares canyon where the highest plutonium and americium activities are found. The samples collected include:

- Four sediment cores (35 cm long and 18.5 cm diameter), sectioned *in situ* into 1-cm layers;
- Two gravity cores (1 m deep) at the same station where, in the previous year, americium heterogeneities were found at a depth of 18 cm;
- Four cores in other zones of terrigenous input along the Spanish coast (Ebro, Barcelona, Valencia and Alicante).

A detailed summary of the sampling campaign has been published in CIEMAT/IMA/UGIA/M5A07/03/92 (in Spanish). The topography of the zone of study in Palomares is shown in Figure 1.

(2) A technical study of the bathymetry in the Palomares canyon zone carried out onboard the *Urania* has been performed. A number of conclusions are worthy of note:

- Our bathymetry corroborates that of the 'Carte bathymetrique de la marge Continentales espagnole de Cabo de Gata a Torrevieja Echell 1/200.000 de 1978' carried out by the Societé d'etudes du gazoduc de la Mediterranée occidentales, Segano (subsidiary of Sonatrach);

- Given the separation of the bathymetric profiles taken by the Urania and the topographic characteristics of the zone, it is not advisable to make a new bathymetric chart. Thus, only the depth profile series are given. The graphs obtained clearly show the presence of two tributaries of the main canyon (in the zone of maximum plutonium concentration) that become one as the depth increases. This channel may be the main path for the physical transport of radionuclides associated with sediments.

- The geophysical profiles produced using Sparker (due to technical problems in the equipment) were insufficient in that they did not permit us to identify the zones of maximum sediment accumulation. The lateral scanning sonar is not precise enough to define the rocks at the bottom.

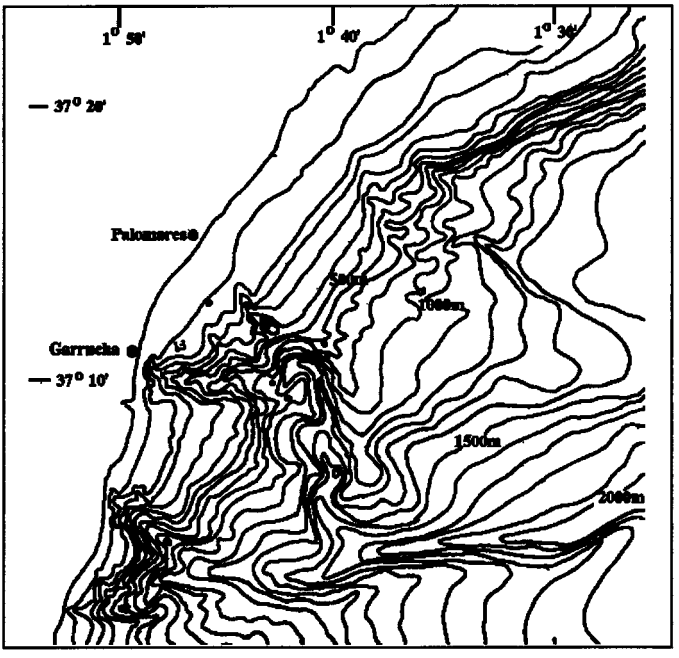

Figure 1. Submarine topography off Palomares

(3) A method of sequential extraction of plutonium in marine sediments has been developed by modifying a previously existing one used in CIEMAT for soil studies. Duplicate aliquots from a standard material (IAEA-367) have been analysed using this technique. The results are given in Table 1.

The results indicate that the addition of all the fractions (available, exchangeable and specifically adsorbed, associated with organic matter, associated with sequioxides, and residual) is identical to the certified values for the sample and that there is no statistically significant difference between duplicates.

Table 1. Sequential leaching of plutonium in sediments (IAEA-367)
The certified activity of $^{239,240}Pu$ is 38 (Range 34.4 - 39.8) Bq kg^{-1}

Fraction	Pu concentration (Bq kg^{-1})		% in sediment	
	Aliquot 1	Aliquot 2	Aliquot 1	Aliquot 2
Available Pu	0.01 ± 0.01	0.01 ± 0.01	0.03	0.03
Exchangable and specifically adsorbed Pu	0.16 ± 0.04	0.12 ± 0.03	0.43	0.38
Pu associated with organic matter	9.51 ± 0.78	7.41 ± 0.61	25.70	23.64
Pu associated with sequioxides	19.87 ± 2.68	16.75 ± 2.47	53.43	53.43
Residual Pu	7.66 ± 1.55	7.06 ± 0.53	20.59	22.53
Total	37.21 ± 3.19	31.35 ± 2.59	98 ± 9	83 ± 8

[All uncertainties quoted are ± 2 S.D.]

The statistical analysis between duplicates has been done using the following formula:

$$S_D = \sqrt{\frac{\sum_i d_i^2}{2P}}$$

The results are given in Table 2.

Table 2. Statistical analysis of the duplicates

Duplicates		Mean	D	d_i	d_i^2
0.01	0.01	0.01	0	0	0
0.16	0.12	0.14	0.04	0.285	0.081
9.51	7.41	8.46	2.10	0.248	0.061
19.87	16.75	18.31	3.12	0.170	0.029
7.66	7.06	7.36	0.60	0.081	0.006
			X = 5.86		Σ = 0.178

$$S_D = \sqrt{\frac{0.178}{10}} \; x \; 100 = 13 \; \%$$

The duplicates are statistically identical at a confidence level of 87%.

(4) The first results of the analysis of Pu in the terrigenous input of the Ebro river show a high sedimentation rate and an homogeneous concentration of plutonium in all the sediment layers analysed (13 cm). The concentrations found in the profile are given in Table 3.

Table 3. Transuranic concentrations and inventories in an Ebro river mouth sediment core (*Station:* URTARSD12, *Coordinates:* 40° 45.11´ 1° 04.92´E, *Depth:* 98.6 m)

Section (cm)	Activities in Bq kg^{-1}			Inventories in Bq m^{-2}	
	^{239,240}Pu	^{238}Pu	^{241}Am	^{239,240}Pu	^{238}Pu
0-1	0.66 ± 0.08	0.036 ± 0.014	0.15 ± 0.11	5.37	0.29
1-2	0.73 ± 0.08	0.033 ± 0.013	0.47 ± 0.21	3.76	0.17
2-3	0.70 ± 0.08	0.059 ± 0.018	0.39 ± 0.26	4.85	0.41
3-4	0.68 ± 0.08	0.040 ± 0.012	-	5.02	0.29
4-5	0.64 ± 0.08	0.027 ± 0.013	0.30 ± 0.07	4.83	0.20
5-6	0.68 ± 0.09	0.033 ± 0.015	-	5.26	0.25
6-7	0.55 ± 0.08	0.041 ± 0.018	0.40 ± 0.33	4.28	0.32
7-8	0.86 ± 0.13	0.036 ± 0.023	-	6.90	0.29
8-9	0.62 ± 0.06	0.035 ± 0.013	-	6.60	0.37
9-10	0.30 ± 0.04	0.018 ± 0.009	-	3.30	0.20
10-11	0.43 ± 0.07	0.018 ± 0.013	-	2.82	0.12
11-12	0.18 ± 0.03	0.023 ± 0.012	-	2.13	0.27
12-13	0.09 ± 0.06	0.024 ± 0.036	-	0.59	0.16
				Σ = 55.7	Σ = 3.28

[All uncertainties quoted are ± 2 S.D.] [- Not analysed]

(5) The CIEMAT marine radioecology laboratory participates in all the intercomparison exercises organised by the IAEA on samples of marine origin. For example, duplicate samples of the intercomparison materials IAEA-300 and IAEA-315 are presently being

analysed for their plutonium, americium and other radionuclide content. The results of these analyses will be reported in due course.

Scientific team

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Gascó Leonarte, C., Antón Mateos, M.P. and de Andrés, J.R. (In Press),'Distribution of long-lived radionuclides along the Spanish Continental Shelf affected by terrigenous river contribution. Study of the Palomares Submarine Canyon', In: MED'92 Cruise Report, R. Delfanti and C. Papucci (Eds.), ENEA, La Spezia.

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Head of project 3: Dr. Guéguéniat

Objectives for the reporting period

(1) To study the pathways of plutonium associated with deposited sediments of megatidal areas, with special attention to the Channel;

(2) To study the behaviour of dissolved and particulate plutonium isotopes in the vicinity of the La Hague Plant, on the French coast of the Channel and in the Seine estuary;

(3) To elucidate the mechanisms of americium complexation by experimental studies.

Progress achieved including publications

(1) Pathways of plutonium associated with deposited sediments of megatidal areas

Considerable progress has been made in this field of investigation. Plutonium analyses have been carried out on bulk sediments and less than 50 μm fractions, assumed to be the most mobile in high-energy sedimentation areas. Forty sub-tidal sediment samples were analysed in the French side of the Channel, from Brittany to the Straits of Dover, including the Roads of Cherbourg and the outer part of the Seine Estuary. In addition, two box cores (off Texel and Hamburg) were studied to asses mixing processes between sediment particles originating from the Channel (La Hague) and from the Irish Sea (Sellafield). We will review, hereafter, the main features of plutonium distribution in some typical sedimentary environments.

On the *North Coast of Brittany*, two samples of fine-grained sediments were analysed, from Aber Vrach and Roscoff; despite the considerable amounts of clay particles (probably more than 50% of the total), ^{239,240}Pu and ^{238}Pu concentrations are only 1.07 - 0.04 and 0.47 - 0.03 Bq kg^{-1}, respectively, while ^{238}Pu/^{239,240}Pu activity ratios are lower than 0.06. These values are in the same range as those measured in the Gironde Estuary and enable us to conclude that no industrial impact is likely to be detected at these sites.

In the *Norman-Breton Gulf*, plutonium analyses were performed on bulk sediments and less than 50 μm fractions; in the former, ^{239,240}Pu concentrations are in the range of 0.4 - 3.3 Bq kg^{-1} (5 samples) and are clearly correlated with the abundance of clay (estimated by Al measurements); in the latter, ^{239,240}Pu concentrations are higher and more homogenous, as expected (3.2 - 6.0 Bq kg^{-1}; 10 samples). Activity ratios, as defined above, are typically 0.4 - 0.5 in the eastern part of the area and tend to increase northwards (0.55 - 0.69 values observed off Jersey). In contrast, western points (Bay of Saint-Brieuc) and/or coarser-grained sediments display lower activity ratios (down to 0.2).

In the *vicinity of Cherbourg*, investigations have been continued on both cores and surficial sediments; two cores have been especially studied; ^{210}Pb excess measurements gave evidence of continuous sedimentation throughout the 30 past years at sedimentation rates of *ca.* 3.0 - 0.1 cm y^{-1}. Consequently, each depth interval has been dated down to the bottom of the cores, that is to say, to the early sixties. At this time, the ^{239,240}Pu concentrations did not exceed 4 Bq kg^{-1}, and isotopic ratios ranged between 0.03 and 0.07. The first industrial impact in the sedimentation record of this area is observed in the early seventies. Two peaks in ^{239,240}Pu concentrations are recorded in the cores corresponding to 1977 and 1985 (29 and

46 Bq kg^{-1}, respectively). Only the former peak is observed in the ^{238}Pu activity profile (19 Bq kg^{-1}). In contrast, the $^{238}Pu/^{239,240}Pu$ activity ratio displays a continuous evolution with time, starting at 0.05 in 1973, reaching 0.85 in 1990, and nearing 1 in 1992. We believe that the plutonium activities recorded in the fine-grained sediment of the Roads of Cherbourg provide a reliable record of the particulate plutonium fluxes, originating from the southwestern Channel and passing eastwards, at the meridian of Cherbourg. This is a key observation for further studies of particle time transits (see below).

In surficial sediments (1992 cruise), the activities range between 2 -14 Bq kg^{-1} depending on grain-size distribution of the sediments, in first approximation. Activity ratios depend on granulometry, but also on sediment processes occurring in the roads yielding the admixture of particle pools of different 'ages' ('age' is to be understood here as time elapsed since labelling by the La Hague effluent).

In the *Bay of Seine* and, more generally, in the eastern Channel, interesting trends are observed in the plutonium sediment distribution which can be summarised as it follows:

- activity ratios decrease from Cherbourg to the Dover Straits for a given granulometry;
- a given activity ratio is found closer to Cherbourg, if it is measured in a coarser-grained sediment.

When combined with the quasi-linear evolution of activity ratios in particles versus time, observed in Cherbourg, these data enable an estimate to be made of the apparent 'age' of sediment admixtures, that is to say, transit times from Cherbourg. The average velocity of sediments depends on their grain-size distribution; for example, sandy sediments collected in the Central Channel, north of Cherbourg, have an average velocity of *ca.* 13 km per year, while for fine-grained sediments, an apparent velocity of 40 km per year has been estimated.

These preliminary (and promising) results are yet to be enriched, and mixing models of sediments of different age and origin need improvements; nevertheless, they provide evidence that the analysis of plutonium isotopes is a valuable tool with which to study particle transfers and, consequently, to find out the pathways of particulate plutonium in high-energy sedimentation areas.

(2) Behaviour of dissolved and particulate plutonium isotopes in the vicinity of the La Hague plant, on the French coast of the Channel and in the Seine Estuary

In *nearshore waters of the Cotentin*, $^{239,240}Pu$ or ^{238}Pu concentrations vary between 10 and 30 μBq l^{-1}; the activity ratios ($^{238}Pu/^{239,240}Pu$) are higher than 1, and can reach 2, which is consistent with what is known of the effluent from the plant. In contrast, the activity ratios of associated particles (0.5 - 3 mg l^{-1}) are lower than 1 (0.4 - 0.8) and are thought to be influenced by 'older' sediments resuspended from the Normand-Breton Gulf.

In the *Seine estuary*, preliminary results have been obtained from a campaign performed in March 1993, during which samples were collected at different salinities (27, 13, 8, 5, 1.5, 0.4 g l^{-1}). Preliminary measurements surprisingly indicate that dissolved plutonium concentrations are in the range 5-10 μBq l^{-1} ($1.3 < ^{238}Pu/^{239,240}Pu < 2.0$) and do not display significant trends downstream. Another campaign, planned for October 1993, should provide more information on this feature. The associated particulate phase displays low concentrations (1 - 3 Bq kg^{-1} for $^{239,240}Pu$; 0.5 - 1.5 Bq kg^{-1} for ^{238}Pu). Here again, almost no downstream

evolution is observed; furthermore, the activity ratios are in the range 0.33 - 0.50.

Publications

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Head of project 4: Dr. Papucci

Objectives for the reporting period

The overall objectives of ENEA's contribution to the programme are a mass balance for ^{137}Cs in the western Mediterranean Sea and the evaluation of the role of different water masses in the horizontal and vertical transport of long-lived radionuclides in the basin.

The specific objectives for the reporting period were:

(1) Determination of vertical profiles of ^{137}Cs in the water column and in sediments of selected areas of the western Mediterranean; and

(2) Evaluation of the ^{137}Cs inventories in water and in sediments, and estimation of partitioning of ^{137}Cs between the two compartments.

Progress achieved including publications

Prior to the present study, the bulk of the ^{137}Cs data available on the Mediterranean related to surface waters. Although data on vertical profiles are essential for estimating inventories and evaluating the mechanisms of physical transport through different water masses, coverage of the Mediterranean with profile stations has been most uneven and very few data exist for stations at depths exceeding 2,000 m. For this reason, a systematic study has been initiated in the western Mediterranean by our laboratory.

The western Mediterranean Sea is characterised by the presence of three different water masses:

(1) Modified Atlantic Water (MAW), in the surface layer (0-200 m);

(2) Levantine Intermediate Water (LIW), imported from the eastern Mediterranean *via* the Straits of Sicily, in the 200-600 m depth range; and

(3) Western Mediterranean Deep Water (WMDW), in the underlying layer.

Sampling points have been selected in the different basins of the western Mediterranean, based on distinct hydrographic characteristics (decreasing imprint of LIW along its path and presence/absence of recently formed dense waters).

Water sampling was performed after CTD casts. Different tracers were used to identify the water masses to be studied: salinity and temperature for LIW and O_2 concentration for recently formed dense waters. Water samples of approximately 100 litres were collected by *Gerhard-Ewing* or *Go-Flo* bottles. Pre-concentration of Cs was carried out onboard by co-precipitation with ammonium molybdophosphate (AMP). The samples were then analysed by gamma spectrometry upon return to our laboratory.

At present, ten vertical profiles of ^{137}Cs, six of which cover depths greater than 2,000 m, were determined on samples collected in 1991 and 1992 in the western Mediterranean, west of Sardinia. During a cruise carried out in June 1993 samples from another four vertical

profiles (two of which are in the Tyrrhenian Sea) have been collected and processed onboard for radiocaesium.

The concentration of ^{137}Cs (Figure 1) generally decreases from the surface to the bottom (from 4 to 1.5 mBq/l). It is apparent that significant activity is usually present at the greatest depth sampled. ^{137}Cs concentration in MAW and LIW is rather homogeneous all over the basin. The mean values are 3.83 $\pm$ 0.52 an 3.19 $\pm$ 0.36 mBq/l, respectively. Only in the station where the strongest LIW imprint was observed (SW of Sardinia), was a higher value (5.20 mBq/l) found in 1991, compared to 1992. This relative maximum may be due to the transport of Chernobyl ^{137}Cs from the more contaminated eastern Mediterranean basin through the LIW, as already evidenced in 1989 by IAEA-MEL. However, since 1992 the LIW entering the western basin through the Sardinia Channel does not carry any Chernobyl signature.

Relative increases in the ^{137}Cs concentration were also found in WMDW, towards the bottom. It is known that, by the action of strong, cold winds, deep water is formed in the northern area of the western Mediterranean basin by convection of near-surface waters, with relatively high concentrations of ^{137}Cs and dissolved O_2. The depth layers with relative increases in ^{137}Cs concentration, are also characterised by oxygen maxima (Figure 2), suggesting that dense water formation is one of the mechanisms responsible for the vertical transport of radionuclides.

When possible, water samples were also collected in the Benthic Boundary Layer. All of these samples show a small increase in ^{137}Cs concentration, compared with that of the overlying layer, presumably due to the presence of materials resuspended from the bottom.

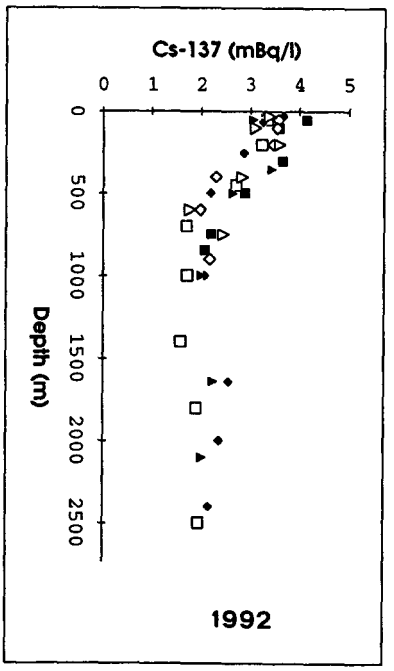

Fig. 1. Vertical profiles of Cs-137 in the water column of the W. Mediterranean Sea.

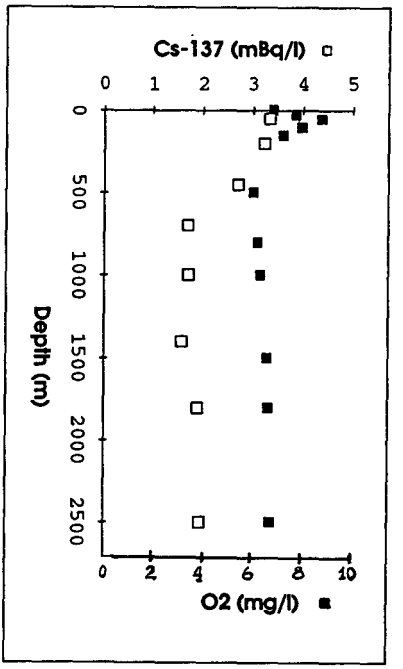

Fig. 2. Vertical profiles of Cs-137 □ and O2 ■ at station 0292 (NW Mediterranean).

For calculating ^{137}Cs inventories in the water column, we have considered two layers. The first one, 0-600 m, where horizontal water motions are dominant, includes MAW and LIW. The second one (WMDW), 600 m to bottom, is a colder and less saline layer, with nearly constant hydrographic characteristics all over the basin.

The inventories of ^{137}Cs in MAW + LIW are rather homogeneous in the western Mediterranean, with a mean value of 1,740 $\pm$ 309 Bq/m^2 (1991-92 data).

The inventories in the WMDW are proportional to the depth of the water column, ranging from 666 Bq/m^2 (828 m) to 4,823 Bq/m^2 (2,770 m).

At present, sediment inventories have been calculated in samples collected in the Sardinian and Balearic Basins, and correspond to 6-9% of the total inventory.

Table 1. Inventories of ^{137}Cs (Bq/m^2) in the water column and in sediments of the western Mediterranean Sea

Year	Station No.	Depth (m)	Inventory MAW + LIW	Inventory WMDW	Total Inventory	Sediment Inventory
1991	0191	1025	2168	1765	3903	233 (6%)
	0291	2800	2042	4725	6767	
	0391	2770	1992	4823	6815	
	0491	1588	1985	2611	4596	
1992	0192	886	1293	1045	2338	
	0292	2800	1647	4049	5696	
	0392	2430	1383	4491	5874	
	0592	931	1862	887	2749	
	0992	2208	1455	3834	5289	
	1692	828	1576	666	2242	228 (9%)

Publications

Delfanti, R., Papucci, C., Salvi, S. and Lorenzelli, R. (1993),'Distribuzione verticale di ^{137}Cs e ^{90}Sr in differenti masse d'acqua del Bacino Algerino (Mediterraneo Sud-Occidentale)', Rapporto Interno ENEA-CRAM, 10 pp.

Delfanti, R., Papucci, C., Salvi, S., Lorenzelli, R. and Bassano, E. (1993), 'Profili verticali di ^{137}Cs e O_2 nella colonna d'acqua del Mediterraneo Occidentale', Rapporto Interno ENEA-CRAM, 15 pp.

Delfanti, R. and Papucci, C., Eds. (1993), MED'92 Cruise Report, ENEA, La Spezia, 121 pp.

Delfanti, R., Frignani, M., Langone, L., Papucci, C. and Ravaioli, M.A.,'The role of rivers in Chernobyl radiocaesium delivery, distribution and accumulation in coastal sediments of the north-Adriatic Sea', Submitted to the International Seminar on Freshwater and Estuarine Radioecology, Lisbon, March 1994.

Delfanti, R., Papucci, C., Desideri, D. and Testa, C.,'Distribuzioni verticali di $^{239,240}Pu$ in sedimenti di mare profundo del Mar Mediterraneo', Accepted for presentation at the meeting: 'La Radioattività ambientale dell'area del Mar Mediterraneo', Isola del Giglio (Italy), May 1994.

Head of project 5: Dr. Woodhead

Objectives for the reporting period

To determine the physico-chemical speciation of radionuclides by size distribution in the Irish Sea prior to the introduction of the Enhanced Actinide Removal Process (EARP).

Specific aims included:

(1) Characterisation of radionuclides in the source term effluent (SIXEP and Seatank);

(2) Examination of the transformation of radionuclide species following dilution of effluents into sea water; and

(3) Determination of the present colloid distribution of radionuclides around the Sellafield outfall and in the vicinity of the muddy area located in the eastern Irish Sea.

Objectives for the next reporting period

Determination of the physico-chemical speciation of radionuclides by size distribution in the Irish Sea following the introduction of the EARP.

A similar strategy of field work and laboratory experiments, to that described above, will be carried out to determine possible changes in the colloidal behaviour of radionuclides in the environment as a result of improvement in waste treatment practices.

Progress achieved including publications

The assessment of the physico-chemical forms of radionuclides in sea water prior to EARP was achieved, using the ultrafiltration technique, on CIROLANA 12/92. In all, five sampling locations were chosen in the immediate vicinity of the Sellafield discharge pipeline (PLZ). These included a southwest transect (incorporating the area of muddy sediment) away from PLZ, and locations north and west of PLZ. Unfortunately, only surface waters were ultrafiltered, due to adverse weather conditions and time restraints, although it is planned that the fractionation of bottom waters will be carried out in a future expedition. For comparative purposes, a further site distant from the Sellafield source term (Scilly Isles) was also investigated. Supporting ultrafiltration trials were carried out to investigate, in more detail, the stability of radiocolloids in sea water with respect to aggregation/dissociation processes due to experimental conditions. Parameters which were investigated included transmembrane pressure, permeate/retentate ratios and duration of processing. For ultrafiltered samples and trial experiments, aliquots were taken and initial chemical separations carried out to determine the concentrations of total $^{239,240}Pu$, $^{239,240}Pu(IV)$, $^{239,240}Pu(V)$ and ^{241}Am in the different fractions. Further purifications and radiometric assays are in progress.

To further assist in the interpretation of possible changes of physico-chemical forms, by the introduction of EARP, a comprehensive survey was also carried out (on CIROLANA 12/92) to determine (i) the present oxidation state distributions of transuranic elements, (ii) the speciation of ^{60}Co, and (iii) the distribution of other radionuclides (^{99}Tc and ^{137}Cs) in the Irish

Sea and beyond. The majority of radiometric analyses have now been completed with the exception of the assay of the transuranic elements.

Laboratory experiments have been carried out to determine the extent of radionuclides present in the colloidal size region in the source term and possible transformations of forms upon discharge into the marine environment. Hence, the determination of physico-chemical forms (by size fractionation) of a suite of radionuclides in low-level Sellafield effluents has been carried out using the ultrafiltration technique. Samples were collected (with no further treatment) from two different effluent streams (SIXEP and Seatank) on a number of occasions and filtered (0.45 μm) to remove suspended particulate material. The filtrates were size-fractionated through hollow fiber cartridges (3K MW cut-off). Results suggest that the abundance in colloidal form of each radionuclide considered was larger in SIXEP effluent than in Seatank effluent, presumably as a result of the different treatment processes. Taking account of differences in radionuclide concentrations and volumes of discharges for both effluent streams, it is clear that a significant amount of ^{239,240}Pu(IV) and ^{241}Am has been discharged into the marine environment in a colloidal form. For the remaining nuclides considered (^{137}Cs, ^{90}Sr, ^{99}Tc and ^{239,240}Pu(VI)) the likelihood of these been discharged in colloidal form was not considered to be significant.

A number of experiments have been performed to determine the persistence of previously identified colloidal forms, including dilution of SIXEP effluent into filtered and whole sea water, and the behaviour of radionuclides associated with clinoptillonite particles and with iron hydroxide precipitates (as found in SIXEP and Seatank effluents, respectively). Data from these experiments are presently been analysed.

Publications

McCubbin, D. and Leonard, K.S. (1993), 'A preliminary study to assess the effect of some sea water components on the speciation of plutonium', *J. Radioanal. Nucl. Chem. Articles* **172**, 363-370.

Leonard, K.S., Harvey, B.R., Woodhead, R.J., Brooks, T. and McCubbin, D., 'Assessment of an ultrafiltration technique for the fractionation of radionuclides associated with humic material', Submitted to *J. Radioanal. Nucl. Chem. Articles.*

Head of project 7: Dr. Sánchez-Cabeza

Objectives for the reporting period

The overall objective of UAB within the framework of the collaboration is to study the transfer of transuranics and other radionuclides through the primary producer route in the food-chain leading to man. Specific objectives for the reporting period were:

(1) To test a large zooplankton fishing net under field conditions, with a view to obtaining large samples for the analysis of transuranics and other radionuclides, and to measure the concentration of plutonium and other gamma-emitting radionuclides in zooplankton from the Catalan Sea and the Palomares area;
(2) To identify the biological species of the plankton samples collected;
(3) To implement in UAB techniques, developed by UCD, to determine the colloidally-bound fraction of transuranics in natural waters in order to contribute to an extension of the Mediterranean database with emphasis on the Catalan Sea and the Palomares area;
(4) To implement in UAB, with the collaboration of UCD, the dual tracer technique (selective co-precipitation on NdF_3) used extensively by the latter, in both the Irish Sea and the Mediterranean, to quantify the chemical speciation of plutonium in sea water, and to measure the plutonium oxidation state distribution in two samples of sea water, one from the Catalan Sea and the other from the Gulf of Vera; and
(5) To determine the concentration profiles and inventories of plutonium and americium in a sediment core from the Palomares area.

Objectives for the next reporting period

(1) To measure the concentrations of transuranics and other radionuclides in zooplankton from the Gulf of Lyons and the Irish Sea, and to complete studies on plankton sampled in the Mediterranean Sea in the course of the first reporting period;
(2) To measure the concentrations of transuranics in the soluble and particulate (>0.22 μm) fractions of water from the Mediterranean Sea;
(3) To study the vertical transport of transuranics in the Catalan Sea;
(4) To determine, in collaboration with CIEMAT and ENEA, radionuclide concentration profiles in selected sediment cores from the Palomares area and the Catalan Sea and estimate radionuclide inventories.

Progress achieved including publications

UAB has participated in a number of sampling campaigns in the period 1991-93 including FE'91 (Institut de Ciències del Mar (ICM), Spain) aboard the *García del Cid*, MED'91 (ENEA, Italy) aboard the *Bannock*, MED'92 (ENEA, Italy) aboard the *Urania*, FE'93 (ICM, Spain) aboard the *Hespérides* and FLUBAL'93 (Universidad de Barcelona, Spain) aboard the *Minerva*.

Zooplankton

Broadly speaking, zooplankton includes all mobile microbial marine species. A widely

accepted sampling cut-off between phytoplankton and adult zooplankton in the Mediterranean Sea is 200 μm, though some zooplankton species are smaller and some phytoplankton species of lesser dimensions will be sampled when the net saturates.

General transuranic concentrations in the Mediterranean Sea are extremely low. Thus, though zooplankton concentration factors are large (10^3-10^6 l kg^{-1}, dry weight), it is still necessary to collect relatively large quantities of sample for analysis. Our 200 μm zooplankton net has a 1 m diameter mouth, is 5 m long and is provided with a *General Oceanics* flowmeter which can measure, with satisfactory precision, the amount of water filtered.

Five zooplankton samples, collected during the MED'92 expedition (Table 1), were analysed for plutonium and gamma-emitting nuclides. The resulting data are given in Tables 2 and 3. Zooplankton biomass was larger at the northernmost stations (Barcelona and Golf de St. Jordi, mean 18 $\pm$ 2 mg (d.w.) m^{-3}), indicating higher productivity due to higher concentrations of nutrients from (i) urban waste (Barcelona) and (ii) riverine input (Ebro river discharges to the Golf de St. Jordi). In the Palomares area, the mean specific biomass was much lower at 5 $\pm$ 2 mg (d.w.) m^{-3}.

Table 1. Main properties of zooplankton samples from the Mediterranean Sea, 1992.

Location	Station	Long. (N)	Lat. (E)	Volume (m^3)	Wet wt. (g)	Wet/dry weight	Biomass (mg dw /m^3)
Barcelona	7	41°15´	2°10´	867 $\pm$ 17	138.43	7.99	20.0 $\pm$ 0.4
Golf St. Jordi	11	40°51´	0°50´	479 $\pm$ 10	95.86	12.48	16.0 $\pm$ 0.3
Palomares	23	37°12´	-1°46´	1882 $\pm$ 38	124.16	8.07	8.17 $\pm$ 0.17
Garrucha	20	37°9 ´	-1°49´	1817 $\pm$ 36	32.29	5.63	3.16 $\pm$ 0.06
P. Macenas	30	37°5´	-1°50´	1435 $\pm$ 29	72.01	10.59	4.74 $\pm$ 0.10

Table 2. Plutonium concentrations in zooplankton from the Mediterranean Sea, 1992.

Location	^{239,240}Pu (Bq/kg d.w.)	^{239,240}Pu (μBq/m^3)	K_d (l/kg) x 10^3	% total activity x10^3
Barcelona	0.38 $\pm$ 0.02	4.9 $\pm$ 0.3	29 $\pm$ 5	0.37 $\pm$ 0.07
Golf St. Jordi	0.62 $\pm$ 0.02	6.9 $\pm$ 0.3	47 $\pm$ 8	0.53 $\pm$ 0.09
Palomares	0.36 $\pm$ 0.01	1.91 $\pm$ 0.08	28 $\pm$ 5	0.15 $\pm$ 0.03
Garrucha	2.14 $\pm$ 0.05	4.51 $\pm$ 0.14	163 $\pm$ 28	0.34 $\pm$ 0.06
Playa Macenas	0.56 $\pm$ 0.02	1.87 $\pm$ 0.07	43 $\pm$ 7	0.14 $\pm$ 0.02

Table 3. Gamma-emitting nuclide concentrations in zooplankton from the Mediterranean Sea

Location	Concentration (Bq/kg)					
	^{7}Be	^{40}K	^{60}Co	^{137}Cs	^{226}Ra	Ex.^{210}Pb
Barcelona	835 ± 36	288 ± 13	5.5 ± 0.9	< 2.53	18.3 ± 1.2	398 ± 19
Golf St. Jordi	1090 ± 180	218 ± 6	< 5.4	7.3 ± 0.7	31.2 ± 1.8	494 ± 15
Palomares	340 ± 140	210 ± 6	< 6.8	< 5.8	7.7 ± 0.1	342 ± 12
Garrucha	< 380	131 ± 6	5.7 ± 0.1	< 3.0	7.5 ± 0.6	151 ± 8
P. Macenas	< 620	177 ± 5	< 6.4	< 5.8	14.0 ± 1.2	222 ± 14

In general, our results confirm that zooplankton efficiently concentrate plutonium and other radionuclides. Excluding the sample from Garrucha, $^{239,240}Pu$ concentrations ranged from 0.36-0.62 Bq/kg d.w., with a mean of 0.43 ± 0.13 Bq/kg d.w. Assuming an average soluble $^{239,240}Pu$ concentration in sea water along the Spanish Mediterranean coastline of 13.1 ± 2.2 mBq m^{-3}, the observed K_d shows little spread, ranging from (2.8-4.7)x10^4 l/kg with a mean of (3.3 ± 0.9)x10^4. These values are similar to those reported for other marine environments. However, the zooplankton sample from Garrucha showed a plutonium concentration about 5 times higher than the others, namely 2.14 ± 0.05 Bq m^{-3}, which corresponds to a K_d of (1.63 ± 0.28)x10^5 l/kg.

Among the observed gamma-emitting nuclides, both the ^{137}Cs and ^{60}Co data are too few to draw any conclusions. It is possible that the ^{60}Co detected off Barcelona is attributable to nuclear industry waste. However, we are unable to explain the presence of ^{60}Co in the Palomares area. ^{40}K concentrations show little variability (mean 188 ± 44 Bq/kg d.w.) but the contrary is observed for ^{226}Ra (range 8-31 Bq/kg d.w.), where the highest concentration is observed in Golf de St. Jordi. This is probably related to ^{226}Ra input from the Ebro river. Due to its short half-life, ^{7}Be was not detected in some of the samples. The highest ex.^{210}Pb concentration, which ranges from 151-494 Bq/kg d.w., was detected in Golf de St. Jordi, while the lowest was observed at Garrucha, in contrast to $^{239,240}Pu$.

As shown in a previous study by CIEMAT of the distribution of plutonium in the Palomares area, the highest plutonium levels are observed near Garrucha. Consequently, it seems clear that the higher plutonium concentration observed in zooplankton from this area can be attributed to the same source. As most zooplankton are inter-zonal, they are likely to concentrate radionuclides close to the water-sediment interface where the levels are higher. This conclusion is supported by the observation of lower ex.^{210}Pb values in the Garrucha sample, which implies a higher degree of sediment cycling. However, the possibility that a 'hot' particle is responsible for the higher concentration observed can not be ruled out entirely. We hope to clarify this point by examining isotopic ratios and by new experiments.

ICM (Spain) has kindly identified the species and number of zooplankton in each sample. The correlation between these data and transuranic concentrations is being examined at the present time. Though higher biomass values are observed at the two northern stations, this appear to have little influence on the activity of plutonium per unit mass of plankton

(excluding the Garrucha sample). As the stations studied are subject to different environmental conditions, our results indicate that a certain equilibrium between zooplankton and the surrounding water exists, which is almost independent of the total biomass present. Though the total activity of plutonium associated with zooplankton is small (<0.1%), the latter's potential as an agent for the remobilisation of plutonium (and other radionuclides) and for the modification of its speciation during biological cycling, should not be underestimated.

Plutonium oxidation states

The technique to separate reduced(III,IV) and oxidised(V,VI) plutonium using the NdF_3 selective co-precipitation technique has been succesfully implemented in our laboratory with the help of UCD, and has been used to determine the oxidation state distribution of plutonium in two surface water samples from the Catalan Sea and the Gulf of Vera (Table 4).

Table 4. Plutonium oxidation states in the NW Mediterranean Sea

Fraction	Catalan Sea 40° 10´N 1° 42´E	Gulf of Vera 36° 59´N 1°32´W
^{239,240}Pu concentration (mBq/m^3):		
Particulate	1.67 ± 0.14	5.6 ± 0.4
Reduced (III,IV)	4.4 ± 0.4	4.6 ± 0.5
Oxidised (V,VI)	10.8 ± 0.7	10.3 ± 0.6
Total Soluble	15.2 ± 0.8	14.9 ± 0.8
% Reduced (III,IV)	29 ± 3	31 ± 4
% Oxidised (V,VI)	71 ± 6	69 ± 5
Total soluble + particulate	16.9 ± 0.8	20.5 ± 0.9
% particulate	10 ± 1	27 ± 2

In both cases, most of the plutonium in filtered sea water is present in the oxidised form, though a significant fraction (*ca.* 30%) is present in the more insoluble, reduced form. These data are in excellent agreement with those reported above for the western Mediterranean by UCD, in their more extensive database.

Colloidally-bound plutonium

We have applied the technique of differential sorption on aluminium oxide, optimised at UCD, in an attempt to determine the fraction of plutonium in colloidal form at two sampling stations in the western Mediterranean, one in the Palomares inshore zone and one in the Barcelona offshore zone (MED'92).

In the Palomares sample, the colloidal fraction was estimated to be 23 ± 9 %, in reasonable agreement with data reported by UCD. However, in the Barcelona sample, there was no colloidal plutonium detected. The reasons for this are, as yet, unclear.

Radionuclides in sediments

A sediment core from the Palomares submarine zone has been analysed for plutonium, americium and gamma-emitting nuclides. The data are given in Table 5 and the radionuclide activity ratios in Table 6. The resulting radionuclide inventories are 274 ± 3 Bq (^{239,240}Pu)/m^2, 4.9 ± 0.2 Bq (^{238}Pu) m^{-2}, 63 ± 2 Bq (^{241}Am) m^{-2}, 465 ± 15 Bq (^{137}Cs) m^{-2} and 1,686 ± 111 Bq (ex.^{210}Pb) m^{-2}. These values will be used, together with those of CIEMAT and ENEA, to help map the distribution of these radionuclides in the Palomares zone.

Measured plutonium isotopic ratios indicate the presence of some 'bomb' plutonium, as the ratio is close to 0.02, rather than the expected global fallout value of 0.04. This is confirmed by the relatively high value of the ^{239,240}Pu/^{137}Cs ratio. The ex.^{210}Pb core profile is complex and is not amenable to dating at the present time. Probable explanations include bioturbation and sediment mixing/slumping. At least one 'hot' particle has been identified in this core, the activity of which has not yet been determined.

Table 5. Radionuclide concentrations in a sediment core from the Palomares zone, collected during the MED'91 research expedition (Station 11, 37°10´N 1°49´W, depth 50 m)

Depth (cm)	^{239,240}Pu	^{238}Pu	^{241}Am (Bq/m^2)	^{137}Cs	Ex.^{210}Pb
1.5	25.0 ± 0.7	0.72 ± 0.06	5.2 ± 0.8	85 ± 7	254 ± 51
3.5	15.5 ± 0.4	0.36 ± 0.03	3.7 ± 0.1	52 ± 4	157 ± 29
4.5	14.6 ± 0.4	0.28 ± 0.03	4.2 ± 0.3	47 ± 4	441 ± 34
5.5	17.6 ± 0.5	0.38 ± 0.03	4.0 ± 0.6	51 ± 5	211 ± 32
6.5	23.5 ± 0.7	0.38 ± 0.03	4.6 ± 0.8	45 ± 4	201 ± 30
7.5	59.0 ± 2.1	1.10 ± 0.09	16.8 ± 0.4	57 ± 5	119 ± 32
8.5	27.1 ± 0.7	0.43 ± 0.03	6.9 ± 0.2	37 ± 3	35 ± 26
9.5	H.P.	H.P.	H.P.		106 ± 29
11	46.2 ± 1.9	0.75 ± 0.07	10.9 ± 0.7	43 ± 6	4 ± 39
12	32.5 ± 1.4	0.37 ± 0.06	4.0 ± 1.4	35 ± 4	158 ± 44
13	12.9 ± 0.6	0.18 ± 0.05	2.6 ± 0.4	13 ± 4	
Total	274 ± 3	4.9 ± 0.2	63 ± 2	465 ± 15	1686 ± 111

H.P. hot particle

Table 6. Activity ratios in a sediment core from the Palomares zone, collected during the MED'91 research expedition (Station 11, 37°10´N 1°49´W, depth 50 m)

Depth (cm)	$^{238}Pu/^{239,240}Pu$	$^{241}Am/^{239,240}Pu$	$^{239,240}Pu/^{137}Cs$
1.5	0.0286 ± 0.0025	0.206 ± 0.032	0.29 ± 0.03
3.5	0.0230 ± 0.0020	0.240 ± 0.009	0.30 ± 0.02
4.5	0.0189 ± 0.0021	0.284 ± 0.021	0.31 ± 0.03
5.5	0.0216 ± 0.0019	0.229 ± 0.032	0.34 ± 0.04
6.5	0.0164 ± 0.0015	0.196 ± 0.033	0.52 ± 0.05
7.5	0.0186 ± 0.0017	0.285 ± 0.012	1.04 ± 0.09
8.5	0.0157 ± 0.0013	0.254 ± 0.012	0.74 ± 0.07
9.5			
10.5	0.0162 ± 0.0016	0.236 ± 0.018	1.08 ± 0.16
11.5	0.0115 ± 0.0018	0.124 ± 0.044	0.92 ± 0.12
12.5	0.0136 ± 0.0040	0.205 ± 0.029	1.02 ± 0.33
Inventory	0.0180 ± 0.0006	0.230 ± 0.008	0.59 ± 0.02
Mean	0.0178 ± 0.042	0.248 ± 0.032	0.39 ± 0.23

Acknowledgements

We wish to acknowledge the support received from ENEA, ICM and UB and, in particular, their kind invitations to participate in various research expeditions. We also gratefully acknowledge the technical and scientific support received from CIEMAT and UCD throughout the period of the present collaboration.

Publications

Molero, J., Sánchez-Cabeza, J.A., Merino, J., Vives i Batlle, J., Mitchell, P.I. and Vidal-Quadras, A. (1993),'Particulate distribution of plutonium and americium in surface waters from the Spanish Mediterranean coast', (Submitted to *Journal of Environmental Radioactivity*).

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Progress Report

Contract: **FI3P-CT920035** **Sector: A22**

Title: Pathways of radionuclides emitted by non nuclear industries.

1)	Köster	RIVM
2)	Germain	CEA-LERFA
3)	Travesi Jiménez	CIEMAT
4)	Ortins	LNETI
5)	García-León	Univ. Sevilla
6)	McGarry	RPII
7)	Dahlgaard	RISØ

I. Summary of Project Global Objectives and Achievements

Global Objectives; emphasized in the first year 1992-1993.

Study, characterisation and quantification of the Po-210 and Pb-210 emitted by the Phosphorous industries into estuaries.

Laboratory studies under controlled conditions of the actual uptake by mussels of Po-210 from effluents of wet (i.e. phosphogypsum) and of thermal Phosphorous plants.

Field studies to determine natural background levels of ^{210}Po and ^{210}Pb in abiotic and biotic materials in estuaries and to determine the causes of their variation on the same and between different locations.

Field studies of abiotic and biotic distribution of radionuclides emitted by phosphate industries into estuaries to look into annual fluctuations.

Studies of soils, crustacea and fish of an inter-tidal marsh area flooded by estuary waters, on which effluents from Phosphorous industries are emitted.

Global Objectives; to be included in the second year 1993-1994.

The effects of food preparation practices on Po-210 levels in the parts consumed by man of molluscs, crustaceans and fish.

Detailed dose assessments of Po-210 by consumption of fishery produce from natural marine waters and from estuarine waters exposed to inputs from stockpiled phosphogypsum.

General dose assessments of Po-210 by consumption of fishery produce either or not affected by enhancements from the Phosphorous industry.

Modelling of the abiotic distribution of radionuclides in the Westerscheldt estuary into which effluents from wet and thermal process Phosphorous industries are emitted.

Achievements in the first year 1992-1993.

(Note: weights, concentrations, etc. are on dry matter basis). The relative bioavailability of ^{210}Po present in effluents was determined in experiments with mussels (Mytilus edulis) in aquaria. These were continuously supplied with a mixture of natural estuary water and effluent, the control treatment with natural estuary water only. Also the effect of the length of the exposure period - 10, 20 and 31 days - on the bioavailability was studied in separate aquaria.

Incorporation of a fraction of the applied ^{210}Po by the mussels is obvious, 4% in case of phosphogypsum and 14% of the thermal effluent (Table 1). The ^{210}Po levels in the soft parts showed a constant rise over the 10, 20 and 31 days exposure periods. As the ^{210}Po was applied at the same constant rate per unit of time, the fraction of the ^{210}Po which was incorporated was constant over time.

In the effluent treatments ^{210}Pb levels in the soft parts of mussels ranged from 20-50

Bq.kg^{-1}, clearly exceeding the ^{210}Pb levels of 4 Bq.kg^{-1} found in mussels in a field study of the Oosterscheldt and the Westerscheldt. This indicates that ^{210}Pb from phosphogypsum and the thermal effluent is incorporated by mussels though to a lesser extend than ^{210}Po.

Table 1. Incorporation and excretion of ^{210}Po by mussels in aquaria supplied with diluted effluents over a period of 31 days.

Treatment; Effluent dilution	^{210}Po applied in 31 d.	^{210}Po in soft parts		^{210}Po in faeces	
		Incorp.[1]	Bq.kg^{-1}	Fraction[1]	Bq.kg^{-1}
Control; no effluent	2 Bq	loss[2]	200	6%	150
P.gypsum; 1000x	29 Bq	4%	410	30%	5600
Thermal effl.; 100x	110 Bq	14%	2190	10%	12300

[1] Fraction of applied ^{210}Po incorporated in soft parts or in faeces.
[2] In the control treatment the mussels lost some ^{210}Po.

In the Oosterscheldt estuary mussels from the same population and with a shell length of 5-6 cm were sampled on 11-02-1993 and on 05-03-1993. The average weights and ^{210}Po concentrations of the soft parts were 1,1 g with 100 Bq.kg^{-1} in February and 0,6 g with 200 Bq.kg^{-1} in March. Of the mussels considered the quantity of ^{210}Po per animal has been practically constant over this month, whereas in that period the Bq.kg^{-1} activity doubled.
The body weight can also have a marked effect on the ^{210}Po concentration within a population sampled at the same time. Viz. in a sample of 40 mussels, with a shell length of 3,5-4,0 cm collected in January 1993 at the Atlantic coast of Portugal, individual specimen were analysed. Over the weight range from 0,4 to 0,1 gram the ^{210}Po increased from 700 to 1500 Bq.kg^{-1} in the soft tissues. These new findings underline the effect that bodyweight can have on the ^{210}Po concentrations and the need to take this into consideration when studying seasonal or geographical effects.
Information about the natural background in mussels and its variation are needed in order to identify possible causes of natural variations and/or to assess if levels are possibly enhanced due to anthropogenic effects. With this in mind an overview was made of the maximum levels observed by each contractor (Table 2). Possible explanations of the maximum levels vary per country, each of them may be correct though none can be proven. Non anthropogenic or natural factors suggested for explanation are: nearness of rivers during periods of high discharge, low weights of mussel soft parts (lean mussels) due to seasonal or local poor growing conditions, high suspended matter loads, possibly ^{210}Po release from granitic rock submerged in seawater.
Clear enhancements of ^{210}Po in mussels or shrimps due to effluents from Phosphorous plants in operation have thus far only be distinguished in the Westerscheldt estuary and at the coast at the border with and to the North of the Rhine estuary (Netherlands).
In the intertidal marsh area near to Huelva and its P-industries locally rather high 50 Bq.kg^{-1} levels of ^{210}Po in grass (Spartina densiflora) were observed. Levels in the soil in this area are around 500 Bq.kg^{-1}, and dissolved ^{210}Po in the estuary water is enhanced. A possibility is that during high tides the leaves take dissolved ^{210}Po up from the river/estuary water, a new phenomenon worth further study.
With respect to ^{210}Po in the abiotic compartment low levels were identified of ^{210}Po/^{210}Pb dissolved (<1 Bq.m^{-3}), particulate (<90 Bq.kg^{-1}), and in bottom sediment (total sample <60 Bq.kg^{-1}, or 150 Bq.kg^{-1} in the particle fraction <63 μm). These levels coincided for

estuaries and/or coastal waters of Portugal, France, Ireland and the Netherlands. In Spain high background levels are observed in upstream river water with a pH <3,5. In all areas the natural background varied apparently with location and time, unsystematically below and above the levels distinguished as low. Consequently small enhancements could not be identified.
In Portugal and France enhancements in the Seine and Tagus estuary occurred within 0,2-2 km from the effluent outfalls, however irregular and spotty. In France the phosphogypsum is emitted at the border Seine estuary/Channel (Atl.Ocean) and is quickly dispersed and transported. In Portugal most of the phosphogypsum is stockpiled on the estuary margin and the emissions are little. This explains the limited distances over which enhancements can be observed.
In Spain effluents from two plants located at Huelva on the margin of the Odiel estuary 9 km from the Atlantic Coast cause enhancements along this stretch. Both in the intertidal bottom sediments in the marsh lands along the Odiel and the fraction dissolved in estuary/river water of the Odiel and Tinto. The last show higher levels in summer possibly due to a decrease in the river flow.
In a previous project it was found that over a distance of 15 km downstream from the outfall at Rotterdam in the Rhine very clear enhancements occurred in the abiotic environment and to a lesser extend in the coastal zone. In the Westerscheldt estuary enhancements were observed due to emissions from plants at Antwerp and Vlissingen.
At this stage of the project the following conclusions seem to mature. In situations where emissions are small or where plants are located on the coast and effluent dilution and dispersion is large and fast clear enhancements can only be observed within 2 km from the outfall. At farther distances enhancements are difficult to distinguish from variations in natural levels. In situations where plants have large emissions on estuaries or rivers at sites of 6 km or more from the coast clear enhancements can be expected unto the coast and possibly even in the coastal zone.

Table 2. Maximum levels of ^{210}Po found in mussels by each contractor.

Country institute	Max. ^{210}Po in mussel $Bq.kg^{-1}$ dr.wt.	Mussel averages		Specific conditions	
		Dry wt. g	Length mm	Month	Location Sub-stratum
Ireland RPII	400	0,25	42-48	March	Dunquin, in the south of Ireland Bare granitic rock
France CEA/ LERFA	700	0,3	44	March	Aber Wrach, sea with nearby river. Granitic.
	700	0,3-0,6	40-44	March/ June	Baie St. Michel high turbidity.
Portugal LNETI	970	0,28	35-40	January	Atl. coast 5 km S. of Tagus estuary. Sandy shore.
Denmark RISØ	250	0,7	45-60	July	West Denmark Muddy Wadden sea.
Netherl. RIVM	200	0,65	50-60	March	Oosterscheldt estuary. Silty bottom.

Head of project 1: Dr. Köster

II. Objectives for the reporting period.

Objectives, emphasized in the first year 1992-1993.

Technical and administrative coordination of the CEC "Pathways" project.
Study, characterisation and quantification of the Po-210 and Pb-210 emitted by the Phosphorous industries into estuaries.
Laboratory studies under controlled conditions of the actual uptake by mussels of Po-210 from effluents of wet (i.e. phosphogypsum) and of thermal Phosphorous plants.

Objectives, for the second year 1993-1994.

Technical and administrative coordination of the CEC "Pathways" project.
Laboratory studies under controlled conditions of the actual uptake by mussels of Po-210 from effluents of wet (i.e. phosphogypsum) and of thermal Phosphorous plants.
Modelling of the abiotic distribution of radionuclides in the Westerscheldt estuary into which effluents from wet and thermal process Phosphorous industries are emitted.
General dose assessments of Po-210 by consumption of fishery produce either or not affected by enhancements from the Phosphorous industry.

III. Progress achieved including publications.

Summary: exposure of mussels to effluents from the phosphate industry causes enhancement of ^{210}Po in the soft parts; apparently not all ^{210}Po in the effluents is bioavailable, under the conditions of the experiment 14% of ^{210}Po from the thermal effluent (HT) and 4% of ^{210}Po from the phosphogypsum (PT) was incorporated by the mussels; the bioavailability of ^{210}Po from the dissolved fraction of phosphogypsum (PD) is not high in comparison to PT or HT ^{210}Po; there is a clear indication that normal levels and the enhancement of ^{210}Po on a Bq.kg^{-1} basis in the soft parts of the mussels are greater in lean than in fat mussels. The last will be investigated in autumn 1993.

Detailed account of studies executed, results and conclusions.

The coordination required considerable, though rewarding efforts. In the coarse of the project three new CEC contractors joined the contract and two East-European institutes obtained an official cooperation contract. Two inspiring contractors meetings were held of which technical views, and overviews were recorded. Working documents were formulated on mussels and on atmospheric deposition of ^{210}Pb, in order to obtain a general working knowledge of all contractors. With regard to the research the focus of RIVM was on the uptake of ^{210}Po by mussels from effluents of the Phosphorous industries.
Based on extensive studies of the abiotic radiological characteristics of effluents from phosphorous industries (Kö-i.p., Pe-92a, Pe-92b), studies were started on the uptake, incorporation and excretion by mussels (Mytilus edulis) of ^{210}Po and ^{210}Pb from diluted effluents. This in scientific and technical cooperation with the Netherlands Institute of Ecological Research at Yerseke, which institute has the experience and infrastructure for studies of mussels. Phosphogypsum (PT) and effluents from a thermal Phosphorous plant (HT) were obtained with support from respectively Rijkswaterstaat (Ministry of Transport and Public Works) and the plant at Vlissingen. The dissolved fraction of the phosphogypsum (PD) has also been studied.
A 12 day orientation experiment was executed to test methods and concentrations at which effluents could be applied without inactivating the mussels (Table 1).
In both experiments of table 1: unfiltered water from the Oosterscheldt was used, with algae (Phaeodactylum tricornutum) added as extra feed; a control, i.e. a treatment without effluent was included; in stocks, aquaria, etc. air was pumped to obtain a motion in the solutions to prevent the sedimentation of suspended matter.

Table 1. Set up of mussel/effluent studies in aquaria of 14 litre.

	Orientation experiment			Main experiment-1		
Effluent	PT	PD	HT	PT	PD	HT
Ratio effluent:water (mass ratio) [1]	0.001 (C) 0.002 (B)	0.01 (C) 0.01 (B)	0.05 (C) 0.1 (B)	0.001 (C)	0.01(C)	0.1 (C)
Effluent/water (l/d) [2]	12	40	12	12	40	12
Growing period (d)	12	12	12	10, 20, 31	31	10,20,31

[1] B = batch method, effluent/water in aquaria freshened every 3 days; C = continuous method.
[2] Continuous flow rate of effluent/water mixture through aquaria with continuous method.

In the orientation experiment the salinity in the aquaria was 3% and the pH ranged from 7,2 to 7,6. Each treatment was executed in a single aquarium containing 20 mussels. Both with the continuous method and the batch method the mussels stayed active during the 12 days at the concentrations used. This confirms the Belgian (Ba-78) and Polish (Pa-75) 3-day-aquaria-experiments, in which mussels died respectively only at phosphogypsum concentrations of 100 and 5 gram per litre or more.
Uptake of ^{210}Po in the soft parts from the effluent was clearly observed with both methods, ^{210}Po levels were also increased in the faeces (Table 2). (Note: faeces and pseudofaeces were collected together and are denoted as faeces).

Table 2. Orientation experiment, ^{210}Po uptake by mussels from effluent/estuary water mixtures, Febr. '93.

Treatment	Continuous-method		Batch-method	
	Soft parts	Faeces	Soft parts	Faeces
	^{210}Po Bq.kg^{-1} dry wt.			
Control	106	240	121	n.a.
PT12	150	4000	168	n.a.
PD12	134	220	141	n.a.
HT12	253	3600	205	n.a.

It was concluded from the orientation experiment to go ahead with the continuous method in main experiment-1; comprising a control and 7 other treatments (Table 1). The 4 treatments with 31 days growing period had 3 replicates, the other 4 treatments 2. Each replicate was executed in a separate aquarium containing 12 mussels.
Previously it was found that upon mixing HT effluent with artificial estuary water without particulate matter 90% of the ^{210}Po dissolves (Pe-92a). In this experiment the HT effluent is mixed with unfiltered estuary water and apparently ^{210}Po interacts with the mineral and/or organic suspended matter as 90-95% becomes part of the particulate fraction. The same occurs with the ^{210}Po of the PD treatment (Table 3).
^{210}Po levels in the soft parts of the fat mussels in the control of the orientation experiment are around 100 Bq.kg^{-1}. This concurs with levels found formerly in the Oosterscheldt estuary (Kö-92a, We-89). Levels at the start and in the control of main experiment-1 are higher, viz. respectively 200 and 250 Bq.kg^{-1}. The different levels (Table 2 and 3) can be

ascribed at least partly to differences in weight of the mussels, viz. 1,1 and 0,7 gram per mussel.

Table 3. Main experiment-1, ^{210}Po uptake by mussels from effluent/estuary water mixtures, March. '93.

Trt.	Mussel soft parts[1]		Faeces[1]		Inflow per aquarium			Outflow
	Bq per aquarium	Bq.kg^{-1}	Bq per aquarium	Bq.kg^{-1}	Bq total	Bq part.	Bq diss.[1]	Bq. part.[2] per aq.
Start	1,9	250	--	--	--	--	--	--
Contr.	1,7	200	0,2	150	2	1	n.d.[3]	0,2
PT10	2,2	310	5,1	4500	11	14[4]	0,1	n.d.
PT20	3,0	360	6,7	5100	19	18	0,1	n.d.
PT31	3,2	410	9,3	5600	29	24	0,1	2,1
PD31	2,2	230	1,2	370	7	7	0,01	3,7
HT10	6,7	870	5,9	12200	33	31	2,7	n.d.
HT20	12,1	1410	6,9	11900	80	75	4,7	n.d.
HT31	17,1	2190	11,4	12300	110	103	6,4	8,9

[1] Treatment averages the standard deviation generally between 10 and 20%. [2] Measurement of 1 replicate. [3] Intermittent sampling of 1 replicate.[4] n.d.= not determined. [5] Irregular result of particulate ^{210}Po in comparison with total ^{210}Po.

Conclusions on the bioavailability of ^{210}Po from effluents and levels in the soft parts of the mussels, based on main experiment-1 unless stated otherwise; incorporation of ^{210}Po in the mussel shell has been neglected:

- Both phosphogypsum and effluent from the thermal plant give rise to an increase of ^{210}Po levels in mussels.
- The uptake did not decline at the concentrations and periods considered. In the PT and HT treatments the applied ^{210}Po was incorporated almost at a constant rate over the 10, 20, 31 day intervals considered. Viz. 4% of the PT and 14% of the HT ^{210}Po, which indicates a clear difference in bioavailability of ^{210}Po of these two different effluents.
- The increase of ^{210}Po levels in mussels from the PD ^{210}Po is negligible or low at the concentrations used, although the PD ^{210}Po shows clearly up in the (pseudo)faeces. In the PD treatment some NaOH was added for neutralisation, in the orientation experiment the formation of an unidentified gel-like substance around the mussels was observed. This side-effect may have disturbed the bioavailability of dissolved ^{210}Po from phosphogypsum in the PD treatment.
- To distinguish the bioavailability of dissolved and particulate ^{210}Po the PD treatment has been executed for comparison with the PT treatment. Under the experimental conditions the dissolved ^{210}Po in phosphogypsum effluent is not or just slightly more bioavailable than the particulate ^{210}Po.
- The incorporation of applied ^{210}Po in the (pseudo)faeces varies over time, 30-45% in the PT treatments and 8-18% in the HT treatments. The differences between PT and HT are in line with the observed lower ^{210}Po bioavailability of PT than of HT. The varying suspended matter load of the Oosterscheldt water may cause the variation in incorporation over time.

- 14% of the applied ^{210}Po in the PD treatment is incorporated in the (pseudo)faeces, which is relatively low. In the PD treatment approximately 50% of the applied ^{210}Po is put out with the overflow, as compared to 10% in the PT and HT treatments. The PD ^{210}Po behaves different possibly due to above mentioned side effects.
- Whether the ^{210}Po not incorporated by the mussels is bioavailable needs further study by exposing mussels again to this ^{210}Po, present in the outflow of the aquaria and in the faeces.
- The results point out that the CF is different for ^{210}Po from PT and HT, that speciation plays a role, and that probably both dissolved and particulate ^{210}Po are incorporated. As no steady state is reached the CF can not be calculated from the data.

Conclusions on the bioavailability of ^{210}Pb based on a limited number of analyses. In the PT10, PT31, HT10 and HT31 treatments ^{210}Pb levels in the soft parts of mussels ranged from 20-50 $Bq.kg^{-1}$. Much lower than ^{210}Po levels in these treatments, but clearly exceeding the ^{210}Pb levels of 4 $Bq.kg^{-1}$ found in the field study of the Ooster- en Westerscheldt (We-89). This indicates that ^{210}Pb from PT and HT is incorporated by mussels though to a lesser extend than ^{210}Po.

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Head of project 2: Dr. Germain

II. Objectives for the reporting period

In the context of the present contract, the Laboratory for Radioecological Studies of the Atlantic Seaboard (LERFA) proposed to carry out a special study of the impact of certain industrial wastes on levels of polonium in the marine environment and to assess the relative importance of this source in comparison with other sources (geological, agricultural). The area under consideration includes the Seine estuary on the Channel coast of France, where industrial producers of phosphate fertilizer have released phosphatic gypsum waste enriched in polonium (see 1990-1992 report for CEC contract no. B 17 0006). However, the last factory of this type to release its waste into the estuary - the Hydroazote company - stopped releases in September 1992. This event offered an opportunity to monitor polonium activities in the estuarine and marine environment and also to ascertain whether industrial releases influence the radioactivity of the environment.

The study involves establishing the variation in space and time of ^{210}Po in several environmental boxes. To this purpose, the LERFA has performed analyses of ^{210}Po activity in seawater and sediment samples as well as in mussels *(Mytilus edulis)*. The area of interest covers the entire Channel coast of France; previous studies have shown that certain zones (e.g. baie du Mont St Michel) have ^{210}Po activities similar to or even greater than those measured in the Seine estuary.

III. Progress achieved including publications

Summary: Samples of mussels, seawater and sediment were collected from the stations, along the Channel coast of France, during three campaigns, in the periods June-July 1992, October-November 1992, March 1993. For the whole set of samples, the levels of ^{210}Po activity lie in the range 130-770 Bq/kg dry weight for mussel flesh, 0.20-2.70 mBq/l for filtered seawater (<0.45 µm), 20-130 Bq/kg dry weight for suspended matter, and 10-120 Bq/kg dry weight for sediments. The enhanced levels of ^{210}Po in the baie du Mont St Michel and, to a lesser extent, in the Seine estuary are confirmed in the present study. However, occasionally high ^{210}Po levels are observed in "background" stations,particularly in March 1993.

To address the objectives outlined above, samples of mussels, seawater and sediment were collected from the stations (Fig.1) during campaigns in the periods June-July 1992, October-November 1992, March 1993 and June 1993 (^{210}Po data from the last campaign are not treated in the present report).

A general pattern emerges for the distribution of ^{210}Po along the Channel coast of France. Generally speaking, the highest seawater activities are recorded in the baie du Mont St Michel (Cancale, Granville and Pirou) and the Seine estuary (Digue nord), with values commonly in excess of 1 mBq/l (activities at Digue nord

were lower during the period 1990-1992) (Table 1). As observed during the previous study, the ^{210}Po levels at Pointe de Moulard are nearly always lower than elsewhere.

Geographical differences are apparent in the analyses of mussel flesh sampled in June-July 1992 (campaign A); to the west of the baie du Mont St Michel, ^{210}Po activities are below 200 Bq/kg dry weight, whereas in the bay itself, they lie in the range 300-550 Bq/kg dry weight. On the eastern side of the Cotentin peninsula, values are lower than 200 Bq/kg dry weight, while in the baie de Seine and Seine estuary, the mean activity is of the order of 200 Bq/kg dry weight. Finally, to the east of the Seine estuary, the activity of ^{210}Po in mussel flesh is less than 200 Bq/kg dry weight. This pattern of distribution can also be seen in the results of the 1990-1992 study. In March 1993, levels were still relatively high in the baie du Mont St Michel, but were also high at several other stations (e.g. Aber Wrach, Luc-sur-Mer, Digue nord, Wimereux and Gravelines). Apart from a few exceptions, ^{210}Po activities in mussel flesh collected during the campaign of March 1993 are higher than in the two previous campaigns.

The ^{210}Po activities of the sediments are in the range 10-120 Bq/kg dry weight.Some characteristics of the sediments were determined,Al_2O_3(Al is indicator of clay content),$CaCO_3$,SiO_2,C,N,P_2O_5.Although these sediments are not all of the same type,it may be remarked that the highest activities are observed in samples from stations where the sediment is fine.The variation with time at each site is generally very small.

In summary, the enhanced levels of ^{210}Po in the baie du Mont St Michel and - to a lesser extent - in the Seine estuary are confirmed in the present study covering the Channel coast of France. However, some local effects probably linked to rainfall and river inputs are capable of producing ^{210}Po enrichment in the environmental boxes under consideration.

In the light of the present set of results, there appears to be no clear correlation between the levels of ^{210}Po recorded in mussels and in filtered seawater; the same applies to the suspended particulates.

As regards the activities of ^{210}Pb in sediments, the measured values are very close to those of ^{210}Po. For samples that have already been measured from the campaigns of June-July 1992 and October-November 1992, the activities lie in the range 12-110 Bq/kg dry weight. The ^{210}Pb activities obtained from samples (sand) taken at Digue nord are among the lowest recorded. The activities measured in mussel flesh are much less than those for ^{210}Po, ranging from beneath the detection limit up to 17 Bq/kg dry weight in samples analysed to date.

The first results of ^{210}Po analyses on shrimps and fish from the Seine estuary (November 1992) are as follows (in Bq/kg dry weight): - shrimp, *Crangon crangon,* complete specimens(soft parts+"skeleton") 449±27, abdomen flesh 86.3±6.7, abdomen carapace 97.4±7.6, cephalothorax(softs parts+"skeleton" 781±69; - fish, *Platichthys flesus*, intestine 297±18, gills 79.1±5.3 muscles 18.1±1.5, skin 39.1±2.6, liver 144±9, gonads 50.3±3.6.

In this preliminary stage of the study, a comparison of results from the Seine estuary and other sectors of the Channel does not yet enable the recognition of an industrial impact on levels of ^{210}Po in the Seine estuary.

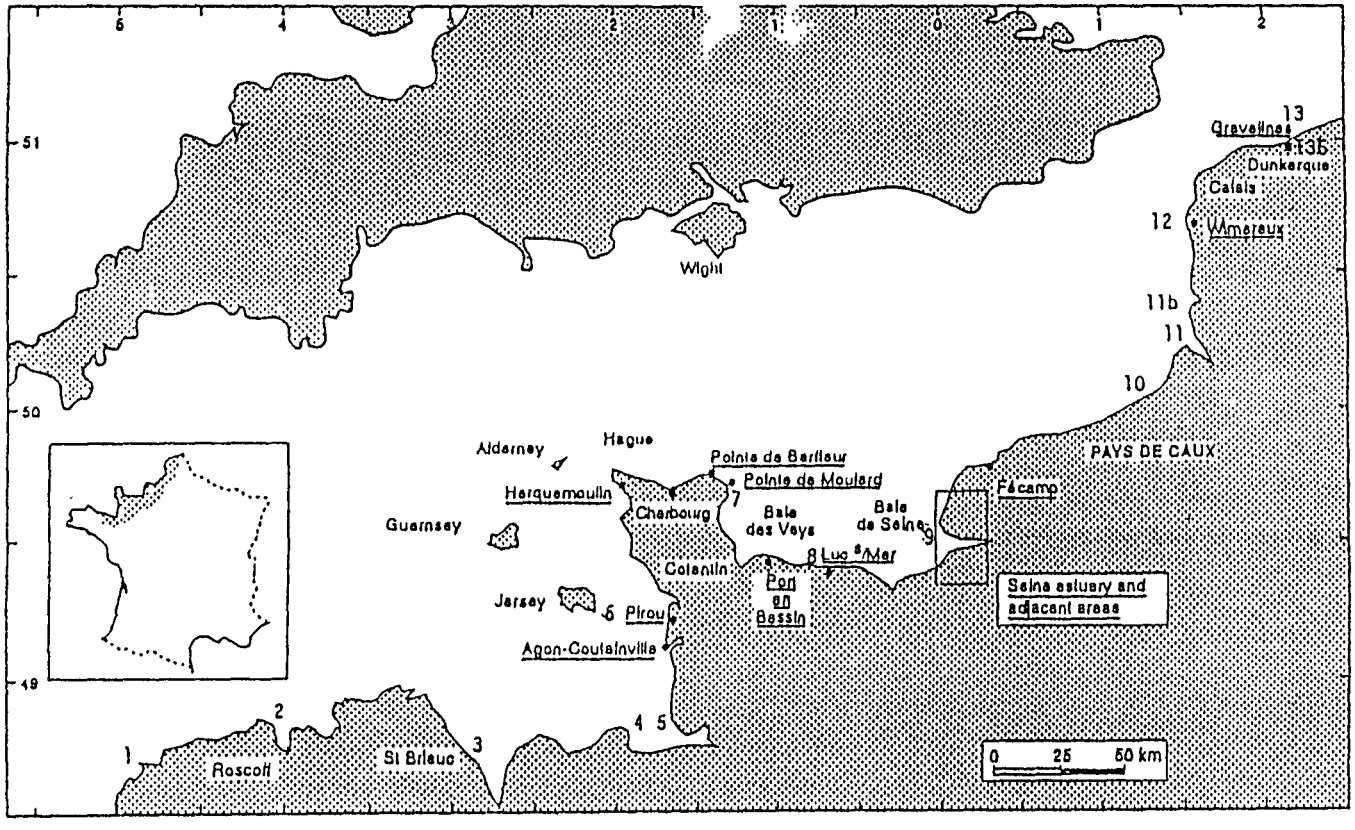


Figure 1 - Location of sampling stations

1 - Aber Wrach, 2 - Roscoff, 3 - St Laurent, 4 - Cancale, 5 - Granville, 6 - Pirou, 7 - Pointe de Moulard, 8 - Luc sur Mer, 9 - Digue nord, Le Havre, 10 - Le Tréport, 11 - Baie de Somme, Le Hourdel, 11b - Le Crotoy, 12 - Wimereux, 13 - Gravelines, 13b - Petit Fort Philippe.

TABLE :
210Po activities measured in Mytilus edulis (flesh),filtered seawater,suspended matter and sediments

stations dates	Mytilus edulis (flesh) Bq/kg dw A	B	C	Filtered sea water mBq/l A	B	C	Suspended matter Bq/kg dw A	B	C	Sediments Bq/kg dw A	B	C
Aber Wrach	153 ± 10 *(0.23)*		772 ± 65 *(0.15)*	0.41 ± 0.03		0.75 ± 0.05	56.6 ± 4.0 (5.6)		58.8 ± 8.6 (35.0)	122.0 ± 8.0		78.9 ± 5.2
Roscoff	185 ± 11 *(0.16)*		276 ± 17 *(0.11)*	1.06 ± 0.06		0.77 ± 0.05	73.9 ± 4.9 (8.5)		112.0 ± 8.2 (37.9)	43.0 ± 3.0		66.1 ± 4.7
St Laurent	196 ± 11 *(0.14)*	307 ± 31 *(0.14)*	234 ± 12 *(0.17)*		1.22 ± 0.09	0.66 ± 0.04		38.7 ± 3.4 (67.2)	40.7 ± 3.0 (39.2)	21.3 ± 1.2	11.5 ± 1.0	59.0 ± 4.4
Cancale	503 ± 26 *(0.17)*	334 ± 28 *(0.21)*	697 ± 31 *(0.22)*	2.05 ± 0.14	2.72 ± 0.20	1.68 ± 0.13	95.8 ± 5.3 (51.0)	73.0 ± 5.0 (14.3)	70.6 ± 5.2 (33.7)	70.2 ± 3.6	79.5 ± 5.9	83.3 ± 5.1
Granville	552 ± 29 *(0.15)*		364 ± 20 *(0.22)*	1.23 ± 0.10		1.37 ± 0.07	20.3 ± 1.3 (31.3)		33.7 ± 2.2 (62.2)	63.5 ± 3.6		85.4 ± 5.9
Pirou	316 ± 18 *(0.18)*	224 ± 15 *(0.25)*	320 ± 17 *(0.32)*	1.74 ± 0.12	0.60 ± 0.05	1.59 ± 0.08	71.4 ± 4.1 (56.9)	68.1 ± 4.8 (93.3)	55.2 ± 4.4 (67.6)			
Pte de Moulard	147 ± 9 *(0.22)*	169 ± 15 *(0.19)*	254 ± 15 *(0.16)*	0.64 ± 0.04	0.68 ± 0.06	0.21 ± 0.02	79.2 ± 8.0 (16.6)	61.9 ± 4.6 (30.6)	34.2 ± 1.8 (16.5)			
Luc/Mer	223 ± 12 *(0.27)*	153 ± 10 *(0.29)*	417 ± 23 *(0.30)*	0.78 ± 0.06		1.66 ± 0.12	65.1 ± 4.7 (39.8)		126.7 ± 8.7 (184.3)			
Digue nord	209 ± 12 *(0.23)*	208 ± 19 *(0.19)*	300 ± 19 *(0.12)*	1.56 ± 0.09	1.48 ± 0.11	1.72 ± 0.13	81.0 ± 13.2 (20.4)	70.6 ± 5.1 (35.8)	101.2 ± 7.0 (29.4)	11.3 ± 0.8	13.3 ± 1.3	17.2 ± 1.3
Dieppe Le Tréport	199 ± 12 *(0.20)*		179 ± 11 *(0.18)*			2.58 ± 0.18			66.5 ± 4.5 (295.5)			
Baie de Somme *	128 ± 8 *(0.13)*	157 ± 10 *(0.23)*				1.38 ± 0.10			64.9 ± 4.8 (111.6)	43.3 ± 3.4	55.3 ± 3.5	55.7 ± 4.3
Wimereux	177 ± 10 *(0.21)*	175 ± 12 *(0.23)*	335 ± 20 *(0.15)*		2.51 ± 0.21	1.38 ± 0.11		60.6 ± 3.8 (107.9)	70.6 ± 5.8 (88.3)			
Gravelines	152 ± 9 *(0.19)*		480 ± 27 *(0.15)*			1.81 ± 0.12			79.5 ± 6.8 (137.5)	57.7 ± 4.2		65.1 ± 4.9

A = June and July 1992
B = October and November 1992
C = March 1993
dry weight/wet weight=(*(0.15)*
turbidity,mg/l=(35.0)
errors are 1 σ
dw=dry weight

* Mytilus edulis.Le Crotoy
seawater and sediments.Le Hourdel

Pathways of Radionuclides emitted by non nuclear industries.

Head of Project 3: Dr. A. Travesi

II. Objectives for the reporting period.

A) Effluents characterization and evaluation.

To characterize the chemical parameters and radioactive compositions of the effluents from the phosphate factories, and the spill from the phosphogympsum piles.

B) Sampling

To select the sampling sites along both rivers Tinto y Odiel and along the common mouth of the estuary formed by them till the Ocean.

To collect bottom sediments samples to be analyzed during the wet season (end of February 1993).

C) Radioactive nuclides in sediments samples.

To determine the Po-210, Pb-210 and Ra-226 activities in a series of 16 sediments samples by radiochemical separations, alfa spectrometry and gamma ray spectrometry respectively in the small grain size ($< 62,5\ \mu m$) sediments particles.

D) Intercomparison test.

To determine Pb-210 and Po-210 in two OIEA intercomparison samples.

III- Progress achieved including publications.

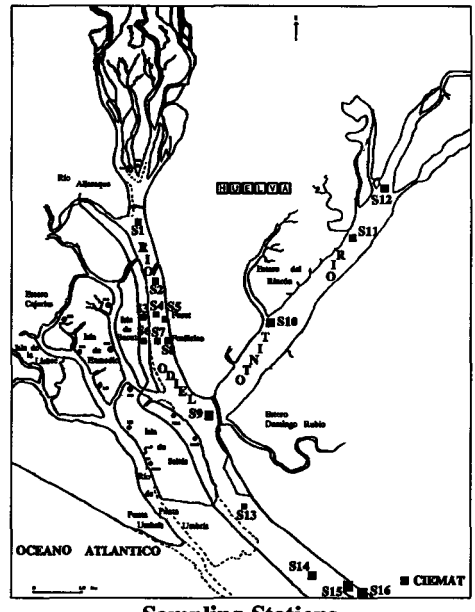
Summary

- The characterization of the effluents samples projected for the "wet season" has not been carried out due to the big "drough" in the South of Spain in the 1993 Spring.
- A series of 16 bottom sediments samples were collected and analyzed for radioactive Po-210, Pb-210 and Ra-226 . Pb-210 activity has been determined by radiochemical methods and by gamma ray spectrometry, both methods showing good agreement.
- Activity levels of Pb-210 in bottom sediments at the **Odiel River** shows:

a) There are appreciable activity levels upstream industrial effluents discard site.

b) The activity level increases at the industrial effluents discard site.The activity level increases strongly at the centre of the river.

c) The activity level at Puerto Nuevo site, downstream the effluents discard site is the same that at upstream sites.



Sampling Stations

Pathways/CIEMAT/IMA

d) The sediments samples at discard site from Fosforico S.A. Factory have a high concentration of total carbon associated to carbonates.

- Activity levels of Pb-210 in bottom sediments at the **Tinto River** shows:

e) The activity levels upstream the discard spills from phosphogypsum piles are natural background concentration.

Table I. Pb-210,Ra-226 and Po-210 Activities in sediment samples

	Pb-210 Bq/Kg		Ra-226	Po-210 Bq/Kg
Sampling Station	Radiochemistry	Gamma ray Spectrometry	Gamma ray Spectrometry	Alfa Spectrometry
S-1	506 ± 31	460 ± 75	406 ± 96	309
S-2	922 ± 38	740 ± 114	584 ± 88	477
S-3	125 ± 21	111 ± 34	< 148	
S-4		2480 ± 168	3395 ± 198	
S-5	231 ± 23	241 ± 50	235 ± 30	194
S-6		103 ± 43	< 241	
S-7	1176 ± 41	1070 ± 169	1140 ± 129	
S-8	254 ± 23	240 ± 40	223 ± 50	212
S-9	763 ± 35	723 ± 111	723 ± 65	
S-10		744 ± 59	6 18 ± 110	
S-11		65 ± 37	< 203	
S-12	31 ± 16	44 ± 13	70 ± 20	39
S-13	445 ± 27	375 ± 59	211 ± 68	
S-14	629 ± 31	595 ± 85	364 ± 77	
S-15	20 ± 15	< 25	< 67	
S-16	< 26	< 24	< 62	

f) Phosphogympsum piles spills increase the Pb-210 activity in sediments **about ten times** over the upstream levels.

g) The grain size distribution and Carbonate composition in the selected samples are very similar. The activity differences can not by attributed to them.

e) At the Atlantic Ocean beaches the levels of Pb-210 activity are again near natural background

Detailed account of activities and findings.

A) Effluents characterization and evaluation.

During this period it has been impossible to take samples of the effluents factories effluents, as projected during the wet seasons (February and March), since exceptionally this year has been a very dry season, and the South of Spain had suffer a big "drought" (sequia).

B) Sampling sites selection and Sample Collection.

Sampling sites have been selected along both rivers **Tinto y Odiel** and along the common mouth of the eestuary formed by them till the Ocean. The sampling sites start at the industrial plants FORET and FOSFORICO in the **Odiel** river and at the phosphogypsum piles in the **Tinto** river. Some samples have been collected upstream these sites in both rivers for control purposes. Other samples have been collected at the Atlantic Ocean Coast near the estuary mouth. Figure 1 shows a map of the area with the sampling stations location.

A series of 16 samples of bottom sediments has been collected at these selected sites by the University of Seville team, since their geographic proximity, wide knowledge and previous experience in this scenery for similar research.

Table II. Grain Size distribution of the fraction < 63. μm

Grain Size Distribution				
Grain Size (μm)	Percentage in Sample (%)			
	S-4	S-6	S-11	S-10
0 - 17	1.7	5.9	3.9	9.2
17 - 34	3.5	10.2	10.8	11.2
34 - 63	5.6	11.3	15.0	10.3

C) Determination of radioactive nuclides in sediments samples.

The measurement of **Po-210, Pb-210 and Ra-226** activities in bottom sediments samples has been carried out by radiochemical separations, alfa spectrometry and gamma ray spectrometry respectively in the small grain size (< 62,5 μm) sediments particles for standardization. Results are shown in Table I.

Four bottom sediments samples have been selected at the effluents discard sites (S-4, S-6) and at the spills of phosphogypsum piles (S-10,S-11), for grain size distribution studies, total carbon concentration, carbon associated to organic-matter and carbon associated to carbonates.

Table II gives the grain size distribution in percentage for grain size interval (μm) for this samples. Also Table III shows the percentage in total carbon, carbon associated to organic-matter and carbon associated to carbonates determined in these samples.

D) Intercomparison test for Pb-210 and Po-210 in OIEA standards OIEA-134 and OIEA-135 has shown good agreements with other participants laboratoties in sediment samples

Table III. Percentage of different types of Carbon.

Percentage of total carbon, carbon associated to organic-matter and carbonates			
Sample	Total carbon (%)	(%) C_{or} organic-matter	(%) $CaCO_3$
S-4	0.74 ± 0.04	0.59 ± 0.03	1.25 ± 0.01
S-6	7.76 ± 0.46	1.48 ± 0.09	52.0 ± 0.37
S-10	1.80 ± 0.10	1.26 ± 0.07	4.5 ± 0.03
S-11	1.90 ± 0.10	1.03 ± 0.06	7.2 ± 0.05

Head of the project: Dr. Ortins

Progress Report Fernando P. Carvalho

II. Objectives for the reporting period

- Concentrations of ^{210}Po in bioindicators from the Tagus estuary and coastal zone; baseline study of ^{210}Po concentrations in mussels throughout an year cycle to adress the seasonal fluctuation of ^{210}Po concentration;
- Active bio-monitoring of ^{210}Po in the Tagus estuary in relationship with the influence of phosphate fertilizer industry, through the use of transplanted mussels;
- Survey of radioactive contamination of the intertidal zone near the stockpiles of phosphogypsum on the Tagus margin.

Objectives for the next reporting period: completion of the study on the seasonal fluctuation of ^{210}Po concentrations in bioindicators; comparison of ^{210}Po concentrations in estuarine and coastal sea species and implications on dose to man through consumption of sea food.

III. Progress achieved including publications

Summary - Concentrations of ^{210}Po and ^{210}Pb were determined in bioindicator species (*Mytilus*, *Balanus*) from the Tagus estuary and adjacent coastal sea. Those concentrations vary widely over time and space, rendering difficult to identify enhanced concentrations of ^{210}Po and ^{210}Pb related with phosphatic waste discharges. Research on the natural concentration of ^{210}Po in mussels and seasonal fluctuation of ^{210}Po concentration was initiated to better define baseline levels in the coastal sea. A similar study is carried out in mussels transplanted into the estuary to assess the bioaccumulation of ^{210}Po from industrial waste releases. A survey was carried out in soils, sediments and plants around the stockpiles of phosphogypsum in the south margin of the Tagus estuary, provided evidence that phosphogypsum seeps into the estuary through surface run off, originating local ^{210}Po concentrations higher that in a reference site.

A wide survey on the concentrations of ^{210}Po and ^{210}Pb in biological indicators was made in samples collected (September 1992) in the Tagus estuary. Mussels and barnacles were collected from the low salinity end member of the estuary through to the coastal sea. Concentrations of ^{210}Po in soft tissues of mussels (*Mytilus galloprovincialis*) ranged from 36 to 510 Bq kg^{-1} (dry wght) and display a trend for increased concentrations with increased salinities. ^{210}Po:^{210}Pb activity concentration ratios in soft tissues of mussels vary between 9 and 57, with a mean value of about 28. ^{210}Po and ^{210}Pb in mussels collected in the middle estuary, near

the zone of phosphatic waste discharges, do not display elevated concentrations in comparison with samples from other estuarine zones. Identical measurements made in barnacles (*Balanus sp.*) collected in the estuary gave ^{210}Po results spreading from 41 to 252 Bq kg^{-1} (soft tissues dry wght) and ^{210}Po:^{210}Pb ratios from 2.5 to 30 with a mean value of about 9. Some of the highest concentrations of ^{210}Po in barnacles were measured in samples of the middle estuary, but do not seem consistently higher than ^{210}Po concentrations measured either in the upper estuary or at the sea entrance of the estuary.

From these results and from other ^{210}Po measurements previously made in mussels in this region, it is verified that ^{210}Po concentrations in the bioindicators species selected spread over a wide range of values and vary widely over time. This variation causes difficulties in the data interpretation and, namely, in the identification of radionuclide concentrations enhanced by releases of phosphatic wastes into the estuary. Furthermore, these difficulties highlight the paucity of present knowledge on baseline concentrations of the naturally-occurring ^{210}Po and ^{210}Pb in mussels, as well as on spatial and seasonal variation of both radionuclides in mussels.

For improving the use of bioindicator organisms in the environmental monitoring of radioactive emmissions from non-nuclear industries, a research programme on ^{210}Po and ^{210}Pb in mussels was started in January 1993. A permanent coastal sampling station was selected in an area with minimal influence of industrial waste releases. Concentrations of ^{210}Po and ^{210}Pb are determined in monthly samples of mussels, sea water and suspended matter. Complementar analyses of chlorophyll a, organic suspended matter and condition index of mussels are carried out.

A preliminar study on ^{210}Po concentration in mussels allowed the determination of the influence of body size of mussels on the ^{210}Po concentration determined. Moreover it was found that large inter-individual differences exist regarding the ^{210}Po concentration in mussels, even when they have nearly identical size (Fig.1). The large intra-specific variation imposes the collection of samples with representative size for radionuclide analyses. For periodic analyses of ^{210}Po and ^{210}Pb in mussels, the mussel size selected is of 3.5-4.0 cm shell lenght and 40 specimens are pooled in a single sample to allow for reduced variation between samples and to enable sufficient resolution for the study on seasonal fluctuaction of ^{210}Po in mussels. Preliminary results obtained since January 93 indicate a strong decrease in ^{210}Po concentration in mussels'soft tissues, from ~ 970 Bq kg^{-1} (dry) in January to ~ 600 Bq kg^{-1} dry in March.

A similar research is being carried out in estuarine conditions through analyses of mussels transplanted from the coastal zone into the estuary, namely into the zone of phosphatic releases. The use of mussels from the same location reduces

the genetic variability and most of the differences in the accumulations of ^{210}Po by mussels can be interpreted as due to variation in ecological parameters. Preliminary results of Po analysis in this transplanted mussels indicate a decrease in concentration over time, approaching Po levels typically measured in estuarine mussels (~ 300 Bq kg^{-1} in March), and thus suggesting the release of its initially high Po content.

A baseline study of ^{210}Po concentration in stickleback fish (*Trachurus picturatus*) was started in January. Through analyses of monthly fish samples a regular decrease in Po concentration in muscle tissue and liver was observed and denotes the wide range in seasonal variation of Po in fish. Results obtained are: ^{210}Po in liver 2800 Bq kg^{-1} in January and 300 Bq kg^{-1} in May; muscle 100 Bq kg^{-1} in January and 23 Bq kg^{-1} in May. Further analyses will generate complementary data to clarify the relationship of Po concentration in fish with Po concentrations in fish prey and in sea water.

To assess the potential introduction of phosphogypsum associated radionuclides into the Tagus estuary by surface runoff, a survey was made in the terrestrial and intertidal environment close to the stockpiles of phosphogypsum disposed off by phosphate fertilizer manufaturing plant on the river margin. Results from this survey indicate that ^{210}Po concentrations in samples of surface soil and surface intertidal sediments (collected within less 200 m from the piles) are much higher than similar samples collected in a reference site in the estuary. Concentrations of ^{210}Po (in < 63 çm fraction) in soils around the gypsum piles vary from 880 to 1085 Bq kg^{-1} (dry) whereas in samples from a reference site they vary between 72 and 100 Bq kg^{-1} (dry). Enhancement of ^{210}Po concentrations in intertidal sediments was also detected: 103-811 Bq kg^{-1} (dry) in the area of phosphogypsum against 27-182 Bq kg^{-1} (dry) in the reference site. Vegetation from the surroundings of gypsum piles also display ^{210}Po concentrations 1.5-6 fold higher than vegetation in the reference site.

Final results of this research programme will allow a better understanding of concentration of naturally-occurring ^{210}Po in the Tagus estuary, namely in bio-indicator organisms, and on the potential of phosphatic wastes for enhancement of environmental concentrations.

References

Carvalho, F.P. (1992). ^{210}Po, ^{210}Pb and uranium in the estuaries of Tagus and Mira rivers (Portugal). Impact of releases from phosphate industry. LNETI/DPSR-A-No.4, III serie, pp.34

Carvalho, F.P. (in prep.). ^{210}Pb and ^{210}Po in sediments and suspended matter in the Tagus estuary. Enhancement of natural levels by wastes from phosphate ore processing industry.

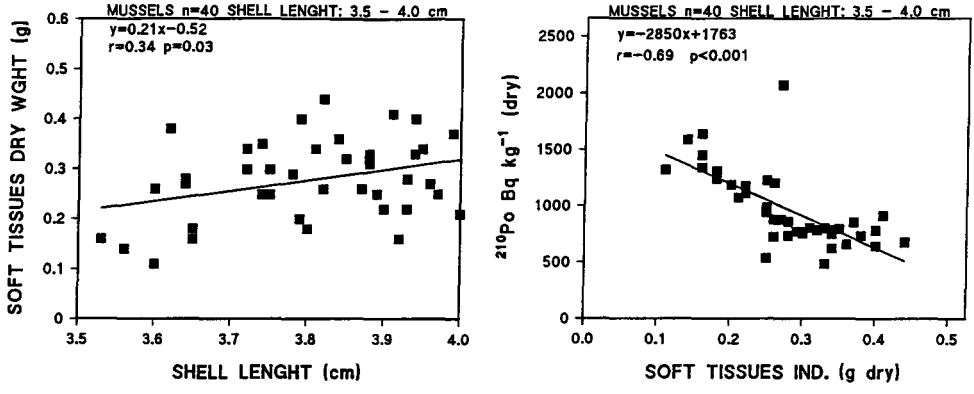


Figure 1. Allometric relationships in mussels (*Mytilus galloprovincialis*) of a narrow size class (Jan. 1993, west coast of Portugal). Left: correlation between dry weight of mussels'soft tissues and maximum shell lenght. Right: ^{210}Po concentration in individual mussels'soft tissues and body size given as dry weight of soft tissues.

First year report of Project 5 (University of Sevilla, Spain)

Scientists involved: A. Martínez-Aguirre, M. García-León

Head of Project 5: Prof. García-León

II. Objectives for the reporting period (Sep. 1992-Aug. 1993)

In previous works the U.S. group has shown that enhanced levels of natural radioactivity (U- Th- and Ra-isotopes) appear in water and sediment samples taken from the estuary of the Odiel and Tinto rivers, Southwestern Spain. In such estuary two phosphate fertilizer factories operate since some 30 years ago, to which such enhancement was attributed.

During the period reported two basic objectives have been planned by the U.S. group: to study the presence of ^{210}Pb(^{210}Po) in water and sediment samples taken at such scenario during 1990 (summer) and 1991 (winter) and to extend to such radionuclides our previous works, and to study the influence of the operation of the mentioned fertilizer factories in the marsh area located at the right margin of the Odiel river, in which a natural reservation exists and an important biological activity takes place (see map).

In addition to that, it was planned (and carried out) a sediment sampling campaign at the Odiel and Titno river beds which were sent to CIEMAT (see its report) for analysis.

III Progress achieved

Summary

To accomplish the above mentioned objectives a sampling campaign was performed during March 1993 (wet season) in the described area. Soil samples (13) were taken and analyzed for ^{210}Po, ^{210}Pb, ^{234}Th, ^{226}Ra, ^{214}Pb and ^{40}K by using α- and/or γ-spectrometry. Spartina densiflora (9) and Spartina maritima (7) samples were also collected during such campaign. They were analyzed for ^{210}Po and U-isotopes so as to gain knowledge on the radioactivity impact on the regional biosphere. Some marine organism samples (sole, ostion shrim) were also simultaneously collected and the analysis are expected to be finished and interpreted by the next reporting period.

Detailed account of findings

1- Odiel and Tinto river waters (samples 1990-1991)

The levels of ^{210}Pb in the Odiel river area under study range from 3.7±0.5 to 289±22 mBq/l with the maxima, in summer and in winter, in front of one of the fertilizer industries. The levels decrease quickly downstream from such a point. This activity pattern is similar to those obtained for other radionuclides in previous works. The levels found in summer are clearly

higher than those found in winter, probably due to a lower water stream flow of the river in the dry season. It seems interesting to compare the data with those obtained in the 1988 sampling campaing for ^{210}Po. The levels in such date were much higher than in 1990 and 1991, with two maxima of 600 mBq/l in front of the fertilizer industries. Such a difference could be related either with a decrease of the effluents from the industries or even with the discontinuity in the discharges. In the case of the Tinto river the levels along the channel are quite constant, ranging from 17.0±1.5 to 26.6±3.0 mBq/l, with a slight decrease downstream the channel. The levels are in this case similar to those found in the 1988 sampling campaing. However, all data seem to be higher than 1 mBq/l given as the background level, reflecting either an enhanced along all the area or that a different background level for South Spain should be considered.

2- Odiel and Tinto rivers bottom sediments (samples 1990-1991)

7 bottom sediments from the Odiel river of each year were separated in four different fractions. The small fraction ($\leq$63 μm) was analyzed for ^{210}Pb determinations in both set of samples. Additionally another fraction (size between 63μm and 355 μm) was analyzed for ^{210}Pb in the 1991 set of samples.

The activity levels in the $\leq$63 μm fractions range from 8.0±0.7 to 799±49 mBq/g in the Odiel river, without differences between seasons. Only in two sampling points (upstream from the industries) the level of ^{210}Pb fall in the non enhanced range (100 mBq/g) with 8.0±0.7 and 40.9±2.7 mBq/g respectively. The rest of sampling points are clearly enhanced during both season. Comparing both fractions analyzed for ^{210}Pb in the campaign of 1991, the same conclusions are extracted as with the analysis of the complete samples, the levels being quite similar.

In the Tinto river the levels range from 33.1±3.3 to 233±9 mBq/g in the $\leq$63 μm fraction for both years. Again, no differences between fractions were found.

In general, no correlations between ^{210}Pb content and organics were found for both rivers, and the activity pattern obtained is similar to those obtained for other nuclides in previous years.

3- Surficial sediments from the Odiel marsh area (samples 1993).

13 surface soil samples were taken in the Odiel marsh zone placed in the right margin of the Odiel river, in front of the fertilizer industries. This area is mainly constituted by four small islands separated by four channels which should be affected by tydal mouvements of the Odiel river. The samples were taken during the low tyde in the zone covered with water during the high tyde. Levels of ^{210}Po in the complete sample as well as in the small fractions ($\leq$63 μm) were determined by α-spectrometry in each sampling point. Moreover a γ-spectrometry measurement of each complete sample was also carried out to determinate the levels of the ^{210}Pb, ^{226}Ra, ^{234}Th, ^{214}Pb isotopes.

In general, by considering the levels of all radionuclides analyzed three differents areas can be distinguished.

One first area can be considered as non enhanced by the fertilizer industries with levels below 50 mBq/g, 100 mBq/g, 20 mBq/g, 200 mBq/g and 30 mBq/g for ^{210}Po, ^{210}Pb, ^{226}Ra, ^{234}Th and ^{214}Pb, respectively. This area covers the further part of the marsh, the west part of the Saltés island and the southwest part of the Enmedio island. A second area presents intermediate levels of each radionuclide: 108 to 163 mBq/g, 150 to 300 mBq/g, 80 to 140 mBq/g, 200 to 223 mBq/g 48 to 60 mBq/g for ^{210}Po, ^{210}Pb, ^{226}Ra, ^{234}Th and ^{214}Pb respectively. This area is no clearly enhanced but the levels are clearly higher than those said above. This area covers the northwest part of the Enmedio island and the east part of the Saltés island. Finally, an area clearly enhanced by the industries with 264 to 367 mBq/g, 480 mBq/g, 300 mBq/g, 400 mBq/g, 100 mBq/g for ^{210}Po, ^{210}Pb, ^{226}Ra, ^{234}Th and ^{214}Pb respectively. This area covers the Mojarrera channel (east of Enmedio island and west of Bacuta island) and a point in the east of the Saltés island where the maxima were found (M12). The differences in such areas should be related with the influence that the Odiel river has over each area by the flow of the Odiel waters during the tydes.

In general, an excepting the point M12, the maxima levels are coincident with a higher organics content of the sample while no relation with the granular composition can be found. The analysis of ^{210}Po in the small fraction has led to the same conclusions showing, in general, concentrations very similar to those in the complete sample.

$^{210}Po/^{210}Pb$ and $^{226}Ra/^{234}Th$ activity ratios below unity were found for all the studied samples. In the case of the first ratio no clear differences have been found between sampling points and activity levels. For the latter ratio, the lowest values are found in the non enhanced area and the highest in the clearly enhanced area of the marsh. This could be an indicative of ^{226}Ra enhancement by the fertilizer industries.

4- ^{210}Po and U isotopes in Spartina Densiflora and Maritima (samples 1993)

In general the levels found for Spartina Densiflora show the same conclusions as those found above with the soil samples. Thus, excepting two points of sampling, the ^{210}Po levels range from 9.4±0.4 to 26.5±1.1 mBq/g. These two excepting points presents the maxima levels with 66.9±3.6 mBq/g and 60.7±3.2 mBq/g which were collected at sampling points M0 and M4. In the case of ^{238}U the levels range from 7.1±0.7 to 13.7±1.1 mBq/g, excepting three samples. These three samples have the maxima leves with 81.0±6.3, 27.4±2.4 and 20.4±1.8 mBq/g in sampling points M0, M12 and M4 respectively. Thus, in general, high concentrations in plants are coincident with high content in soils, as could be expected. The maxima levels (in ^{210}Po and U isotopes) is found, anyway, in the North Marsh, where one Spartina Densiflora was taken in position M0. This sample also presents high content in Fe, Cu, Zn, Ti, Cr, Pt and Ar related to the rest of samples.

Some preliminary U-isotopes results have been obtained for Spartina Maritima (7 samples). Although being early for interpretation, it seems that the activity are higher than those obtained for Spartina Densiflora, but the distribution pattern is similar. It is expected to gain more knowledge for the next reporting period.

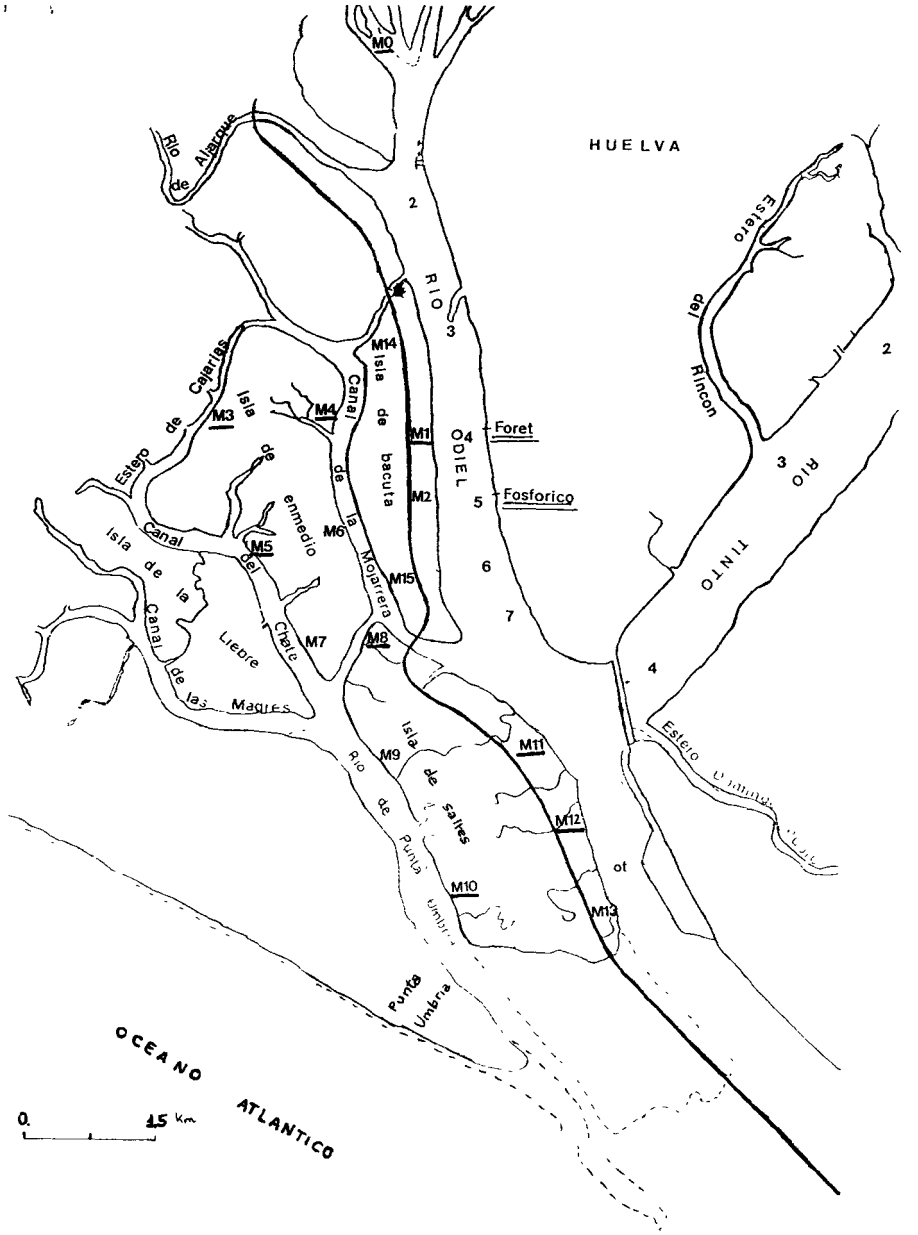


Figura 1: Map of the estuarine system formed at the common mouth of the Odiel and Tinto rivers, including the marsh area. The soil sampling points are denoted by M. Plants samples were taken at soil sampling stations underlined. The phosphate factories are located at "Foret" and "Fosforico" in the left margin of the Odiel river and near the town of Huelva.

Pathways Interim Report

1. Review of RPII Objectives for the Reporting Period.

1.1. Technique Development Phase I: It was necessary to put in place a method for the measurement of polonium in mussels, sediment and seaweed.

1.2. Geographic Distribution Study: It was proposed to implement a programme of mussel sampling and analysis to develop the profile of Po-210 activity concentrations in mussels at a wide variety of locations throughout the coastal regions of Ireland. These locations would include areas where the fertiliser industry was active, where the underlying geology is granitic in nature and pseudo background locations where there are no obvious sources of polonium enhancement.

1.3. Seasonal Variation: It was proposed to identify a suitable sampling location for repeat mussel sampling and analysis over the period of the project to observe any seasonal fluctuations in Po-210 concentrations.

1.4. Dosimetric Studies: It was proposed to implement a sampling programme to assess the dose from polonium to the average Irish fish and shell fish consumer.

1.5. Coastal Water Characterisation: It was proposed to initiate a characterisation of the coastal waters which would include the analysis of mussels, sediment, seaweed and in the second reporting period, water, at a number of locations of interest. Additional analyses were also to be carried out which would include: Nitrate, Phosphate, Silicate, Salinity, pH, Suspended loads, Organic Fraction, Dissolved Fraction, Sediment Size Fractionation analysis, cadmium analysis and ambient dose rate measurements.

2. Achievements At RPII Within Reporting Period

2.1. A technique has been set up to measure polonium in mussels, seaweed and sediment. It makes use of micro-wave digestion systems and the spontaneous deposition of polonium on silver. A polonium-209 yield tracer was used and this was calibrated against a well known Lead-210 standard. The efficacy of the technique was tested and confirmed with samples of established activities and IAEA Intercomparison samples.

At the beginning of the analysis programme an unambiguous quality control regime was put in place. A reagent blank and a quality control sample were budgeted for analysis with every 10 project samples as were systematic instrument performance analysis.

It is proposed that the estimation of supported Polonium be made by determining the Lead-210 fraction using a LOAX Spectrophotometer. The calibration of this instrument is continuing.

2.2. A wide geographical spread of sampling locations were identified on the basis of the aforementioned criteria (Figure 1.). Polonium analysis has been completed on all but one mussel samples collected in the reporting period All data, however await correction for supported polonium. The uncorrected data is presented in Table 1.

2.3. A mussel bed in Sutton (Dublin) has been identified for repeat sampling analysis to obviate seasonal variations in polonium concentrations.

2.4. Fish samples of the most widely eaten species in Ireland have been sampled from the major landing ports as have a number of shell fish species in order to evaluate the average dose from polonium-210 to the Irish consumer. Analysis will be carried out in the second reporting period.

2.5. Mussels, sediment and seaweed samples were taken at 24 locations throughout the country for polonium analysis as part of a coastal water characterisation project (Figure 1, Table 1). Additional environmental parameters were measured at each of the sites as outlined in Section 1.5.

3. Discussion

As measured activities have not, as yet, been corrected for the decay of unsupported polonium, it is too early to present detailed correlation analysis between the various environmental parameters recorded in the project so far However some general observations can be made.

1. No significant increase in polonium concentrations in mussels is observed in areas where the fertiliser industry has been active (Cork, Belfast) as opposed to those areas where no such Industry existed (Carlingford, Kellystown and Dunquin).

2. The highest activity observed was from a pseudo background sample taken at Dunquin, a location far from the fertiliser industry. However the ambient radiation level in this area was higher than in any other sample location and may have an influence on the concentration in mussels. Also there may be biometric parameters influencing the high activity.

Principle Sampling Locations in Ireland

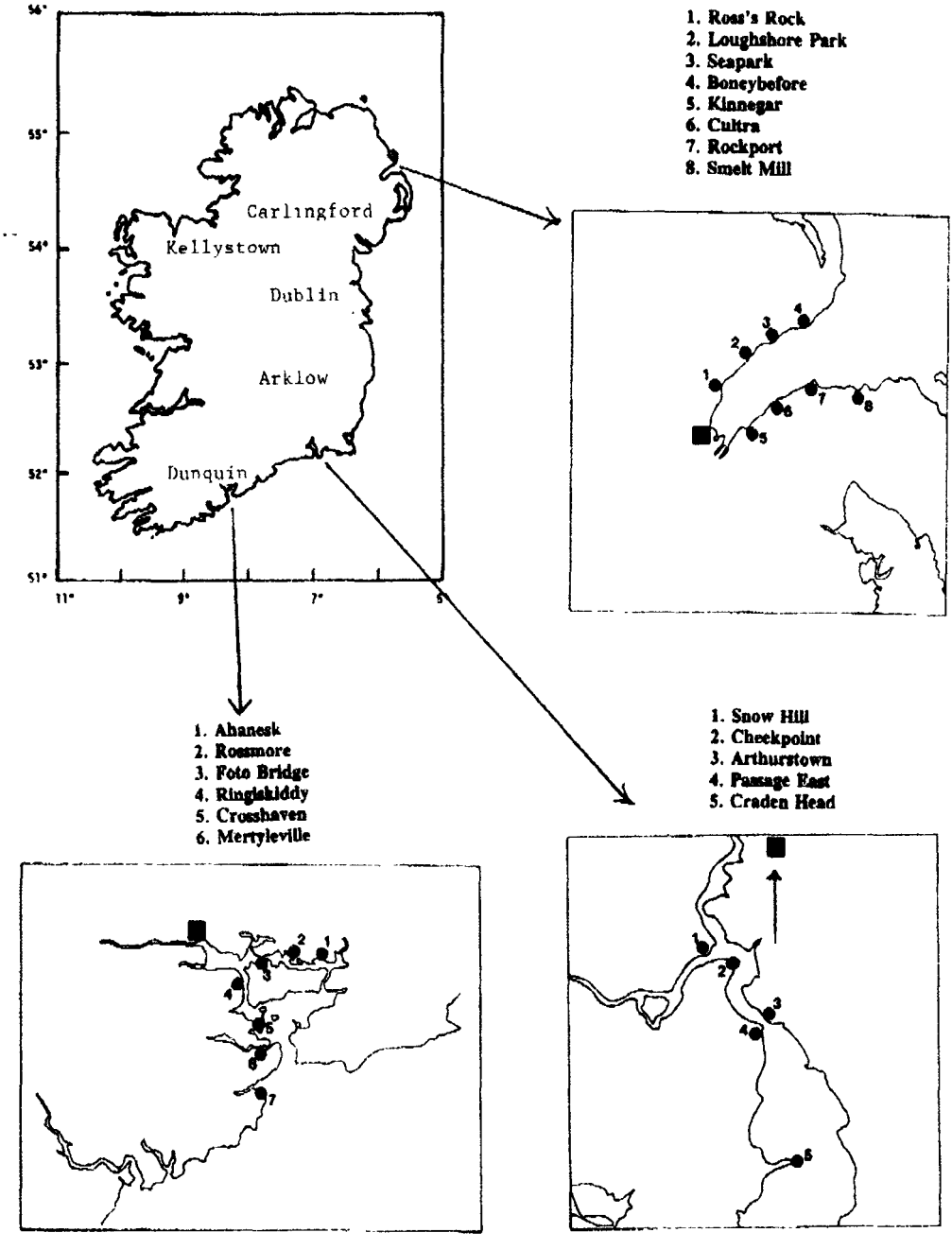


Table 1: Uncorrected Polonium-210 concentrations in Mussels, Sediment and Seaweed.

LOCATION	Po-210 (Mussels) Bq kg^{-1}	Po-210 (Sediment) Bq kg^{-1}	Po-210 (Seaweed) Bq kg^{-1}
Cheekpoint	163	21.6	10.5
Passage East	153	15.8	NA
Craden Head	204	16.7	6.7
Snow Hill	210	36.2	6.7
Arthurstown	165	13.4	6.2
Foto Bridge	131	58.7	18.8
Ahanesk	206	42.7	12.3
Rossmore	161	53.5	9.9
Riniskiddy	206	53.5	15.5
Glenbrook	142	46.0	55.3
Crosshaven	121	17.6	19.1
Mertylville	352	54.3	18.8
Dunquin	397	NA	11.0
Arklow	174	NA	NA
Sutton (Dublin)	147	NA	NA
Carlingford	94	NA	NA
Ross's Rock	77	NA	NA
Boneybefore	114	NA	NA
Seapark	67	NA	NA
Loughshore	NA	NA	NA
Kinnegar	103	NA	NA
Cultra	66	NA	NA
Rockport	92	NA	NA
Smelt Mill	110	NA	NA

Head of project 8: Dr. Dahlgaard

II. Objectives for the reporting period

- two IAEA intercomparison samples (Dec. 1992)
- three fish species are sampled from 3 different locations two times.
- Mytilus edulis will be sampled from 10 locations distributed over Denmark. The locations thus includes salinities from Baltic (8‰) to North Sea coast (~33‰), and it includes two former sites for Phosphate industries.
- The above mentioned samples will be measured for ^{210}Po by α spectrometry. For ^{210}Pb, the concentrations are expected to be too low for γ spectrometry. The concentration of ^{210}Pb will therefore be measured by the much more sensitive ^{210}Po ingrowth method.

Next reporting period:
- Mytilus edulis from one of the above locations, Roskilde Fjord, will be repeatedly sampled approximately monthly to study the variation with time.
- Mytilus will be sampled from the same 10 locations as June 1993.
- three fish species will be sampled from 3 different locations autumn 1993.
- The above mentioned samples will be measured for ^{210}Po by α spectrometry. ^{210}Pb will be measured by the ^{210}Po ingrowth method.
- Remaining samples from the first year will be analyzed for ^{210}Pb by 4-6 months ^{210}Po ingrowth and α analysis.

III. Progress achieved including publications

Summary

Average values for ^{210}Po concentrations in Danish fish meat are given. A relatively large variation calls for a changed sampling strategy. Data on ^{210}Po in Mytilus edulis from 10 Danish sites indicated no excess ^{210}Po activity from two former phosphate industry sites. The average of all 10 Mytilus samples was 154 Bq ^{210}Po kg^{-1} dry soft parts ± 45% (SD). Dublicate samples from Mytilus homegenate indicated a total analytical error for ^{210}Po of ± 10%.

Activity schedule
- November 1992: coordination meeting in Bilthoven
- December 192: Risø was officially added to the already running program. The contract is still in the process of being signed.
- December 1992: two IAEA intercomparison samples analyzed for ^{210}Po. The results were in good agreement with other participants.
- March 1992 and 1993: Cod, plaice and herring samples were taken from

North Sea, Kattegat and the Baltic and analyzed for ^{210}Po.

- June 1993 we introduced a post-^{210}Po analysis cleaning of traces of polonium from the electrolyzed solution by adding a 4m thin Ag filament to the samples for one night as a pretreatment before ^{210}Po ingrowth for the analysis of ^{210}Pb.
- June/July 1993: sampling of mussels from 10 locations. Analysis of ^{210}Po and preparation for ^{210}Pb ingrowth.
- July 1993: coordination meeting in Dublin.

Polonium-210 in Danish fish.

The important commercial fish species cod, herring and plaice were sampled from 3 parts of the country: the North Sea, Kattegat and the Baltic during March 1991, 1992 and 1993. In each case care was taken to obtain fresh fish with a documented catch date. Small samples of fish meat were analyzed for ^{210}Po as soon as possible after the catch - i.e. in most cases within one month. The values were then decaycorrected with the half life of ^{210}Po, as the amount of ^{210}Pb supported ^{210}Po in fresh fish is supposed to be minimal. Average values are given in Table 1. In 18 cases two independent samples were taken and analyzed separately. A χ^2 test indicated a 30% variance between double determinations, which is much more than normally ascribed to analytical errors. It is believed that a major part of this variation has been caused by inadequate sample homogeneity: the samples were not homogenized before they were split up in two samples. Therefore, the double determinations may be from different fish, although they are from the same catch. The variation thus probably indicates an inherent variation between fish in a specific catch. In future sampling, each sample will therefore consist of a homogenate from several individuals, and double determinations will then indicate the total analytical error.

Table 1. Polonium-210 in fish meat. Average ± SD of n independent catches from Danish waters based on fresh weight.

	Bq kg^{-1} fw	± SD	n	fresh/dry weight
Cod	0.22	± 0.11	7	5.1
Herring	0.61	± 0.54	8	3.2
Plaice	0.93	± 0.97	9	5.1

Polonium-210 in Mytilus from Danish waters

In June/July 1993, Mytilus edulis samples were taken from 10 locations covering different hydrological regions in Denmark from the North Sea, the Fjords, Kattegat, the Belts and the western Baltic. In each case the soft parts were isolated from 20 - 35 individuals and homogenized, before two sub-samples were taken for separate analysis. Each sample were analyzed for ^{210}Po by α-spectrometry. A χ^2 test as mentioned above for the fish samples indicated a variation between double

determinations of 10%. As these double determinations were taken from homogenated samples, they indicate the total analytical error. Table 2 shows the measured ^{210}Po concentrations in Mytilus soft parts alongside mussel soft parts dry weight, average shell length and a condition index.

As a pretreatment to analyzing ^{210}Pb by the ingrowth of ^{210}Po, remaining traces of polonium were removed by stirring the sample overnight with 4 m of a 0.125 mm Ag filament.

The Mytilus locations includes two former phosphate industry sites (Fredericia and Aalborg). The present material indicates no excess activity from these sites. An average of all 10 samples gives 154 Bq ^{210}Po kg^{-1} DM ± 45% SD. Future samplings will aim at contributing to the study of mechanisms explaining the observed variations.

Table 2. Polonium-210 in Mytilus edulis soft parts from Danish waters June/July 1993 on a dry soft parts (DM) basis.

Location		Po-210 Bq kg^{-1} DM ± SD		DM g/indiv	length mm (avg)	Condition DM/l^3 *E+6
1	Esbjerg	292.9	± 15.7	0.601	53	4.0
2	Struer	105.8	± 18.2	1.147	53	7.7
3	Aalborg	156.3	± 5.0	0.646	53	4.3
4	Rude Strand	139.9	± 7.8	0.274	50	2.2
5	Fredericia-N	62.7	± 8.6	0.566	48	5.1
6	Fredericia-S	110.8	± 8.4	0.251	35	5.9
7	Nyborg	107.5	± 3.1	0.270	45	3.0
8	Risø	142.6	± 23.5	0.407	50	3.3
9	Rødvig	179.6	± 1.1	0.282	37	5.6
10	Klintholm	240.4	± 0.7	0.243	41	3.5

Progress Report

Contract: **FI3P-CT930075** **Sector: A22**

Title: Investigation on exposure to natural radionuclides in selected areas affected by U-processing.

1)	Belot	CEA - Cadarache
2)	Roehnsch	BFS
3)	Massmeyer	GRS

I. Summary of Project Global Objectives and Achievements

The joint research work began in January 1993. The objectives envisaged for the period ending May 1994 were: (i) to model the atmospheric dispersion of radon from large area sources with models of different complexity; (ii) to acquire experimental data on a French and a German site; (iii) to test the models by using the field data obtained. The consideration of radionuclide migration in geosphere and hydrosphere, originally planned, was postponed, considering the duration and financial support imparted to the study.

A first coordination meeting was held in Berlin and Lengenfeld on 9-11 March 1993. During this meeting it was precised that the final aim of radon models was to predict the annual mean concentrations. Also, it was decided to keep in mind the ultimate need of predicting average concentrations over long periods of time, even if the models are more easily and accurately tested in experiments extending over short periods of time. The acquisition of experimental data was also discussed and experiments in France (St Pierre du Cantal) and in Germany (Lengenfeld, Saxony) were envisaged and defined.

During the first period of the research, theoretical studies were carried out on the transport of radon from ground level area sources. A mesoscale flow model coupled with appropriate dispersion models was prepared by GRS (project 3) to determine the radon dispersion from and over a complex terrrain. In parallel, simple models were implemented at CEA (project 1) and GRS (project 3) to determine the transport of radon over a relatively flat terrain and calculate the radon concentrations at different time-scales. The predictions from these different models are to be compared with data that are being acquired or will be obtained in the near future from field measurements.

Measurement systems to determine radon fluence rates, meteorological conditions and hourly-averaged radon concentrations were tested by CEA (project 1) in a pilot experiment that occurred on 8-10 June 1993 at St Pierre du Cantal. These systems will be used in a joint experiment to be performed on 12-22 September 1993 at Lengenfeld. The field data then obtained will serve to drive and validate the models already prepared. Average concentration of radon were also measured by BfS (project 2) over a protracted period of time (6 months) using nuclear track detectors placed at different distances around the tailing materials of Lengenfeld. These data will be compared to those obtained from models adapted to the calculation of long-term average concentrations.

Head of project 1: Dr. Y. Belot

II. Objectives for the reporting period

Our team contributes to the joint project by: (i) acquiring field data from measurements on French and German sites; (ii) participating to the development of radon dispersion models and codes; (iii) comparing field data and theoretical predictions.

During the reporting period (1.01.93 to 1.08.93), our efforts have been focused on the acquisition of field data (radon fluence rates and concentrations, meterological surface data) to be used in the process of model intercomparison and validation. A pilot experiment was made at St Pierre du Cantal (France) to test the different acquisition systems and make a rehearsal of the experiment to be made at Lengenfeld (Germany) in Sept. 1993.

In parallel, we participated to the development of radon dispersion models by studying, a new model based on a diffusion formula that seems more adapted to ground-level emission than the usual Gaussian formula. This new model is limited to relatively flat terrain, but is relatively well adapted to ground-level emissions and short-distance transport. It remains nevertheless relatively simple and can be easily used for long-term predictions using the statistics available from any meteorological survey network.

The work to be done in the next months will be centered on measurements at the site of Lengenfeld, in cooperation with BfS and GRS teams. The radiological and meteorological data obtained will be used to run the available models (German and French) and test their predictive capability in the determination of short-term radon concentrations in the near-field of radon area sources.

III. Progress achieved

1) Field measurements for model intercomparison and validation

Determination of fluence rate distribution

In order to determine the characteristics of radon emission in the zone of interest, a mapping is made of the radon fluence rate at different sampling points located at the nodes of a sampling grid of uniform mesh size. The smaller is the mesh size, the better is the description of the radon fluence rade distribution, but this would lead in some cases to a number of samples that would be unmanageable. In practice, the mesh size depends on the surface area to be mapped and on the maximum number of samples that can be easily taken and measured by a team within a few hours (about 100 samples).

The radon fluence rate is measured by direct accumulation of radon in a close container resting on the ground surface. Each accumulator device consists of a 30-liter drum that simply collects radon atoms originating below the surface and prevents their being mixed through the atmosphere. The change in concentration of radon in an accumulator placed over a surface is simply related to the flux through this surface, provided (i) the accumulation time is short compared with the half-life of radon, (ii) the concentration in the accumulator remains low enough in relation to that of the soil gas to prevent appreciable back diffusion and (iii) the presence of the accumulator does not seriously disturb the exhalation process. Practically, after an accumulation time of 2-3 h, a sample of the enclosed atmosphere is taken in an evacuated alpha-scintillation flask. The samples are counted during three minutes on a radon counting system after three to four hours have elapsed to insure daughter product equilibrium. Overall error limit was estimated to be about 20 %. This is generally less than the inhomogeneities found in soils while moving from one place to another.

Measurement of radon concentrations in the atmosphere

In the process of model intercomparaison and validation, we need to compare the theoretical predictions of radon concentration in the atmosphere to the real concentrations actually measured at fixed points on the site. This can be done for different time scales, but we are mainly interested here by the validation of models at a short-time scale during periods of one-hour duration for instance. If models give valid predictions at a short-time scale, their extension to longer time scales using the superposition method will also be valid.

The measurement of radon concentrations at fixed points during periods of about one hour is made with ion chambers of 120 or 12 liters, depending on the activity concentration to be measured. The largest chamber is placed on the roof of a truck, it consists of two cylindrical compartments of 120 liters each, one being closed and the other open for circulation of the air to be monitored. The intensity of the ionization current in the closed compartment gives the gamma radiation, and the difference of the ionization currents measured in the two compartments is related to the radon activity concentration. The evolution of the concentration is recorded continuously during the period of sampling. The detection limits are 8 and 37 $Bq.m^{-3}$ for the large and small chamber respectively.

Meteorological determinations

The average weather conditions during the periods of interest are the wind direction, the wind speed at a given height and the Monin-Obukhov lenght L that characterizes classically the stability of the atmosphere. The values of these parameters are derived from the average profiles of wind speed and temperature. The meteorological data are acquired from sensors placed at different levels on a 7-m mast. The low wind speeds close to the ground surface are measured by sensitive hot film anemometers, and the moderate to high wind speeds at greater elevation by classical cup anemometers. The air temperatures are measured with high precision thermistors shielded by two concentric ventilated cylinders. All the data are recorded over the whole duration of experiment on a data logger / microcomputer system.

By fitting the wind and temperature profiles to theoretical expressions, one obtains the friction velocity u_* and the friction temperature θ_* that characterize the eddy fluctuation of wind speed and temperature in the boundary layer. The Monin-Obukhov lenght can then be calculated by the formula $L = u_*^2 T / \kappa g \theta_*$, where T is the average absolute temperature, g the acceleration of gravity and κ the Von Karman constant. If needed, weather stability classes can be estimated from the stability lenght L and aerodynamic roughness lenght z_0 using curves by Golder that show Pasquill's turbulence types as function of stability and roughness lenghts.

Pilot experiment at St Pierre du Cantal

The above methodology was applied (8-10 June 1993) to a French site where uranium mill residues were deposited in the past. German colleagues from BfS and GRS participated to the pilot experiment to become familiar with the techniques employed. The measurements were carried out under favourable weather conditions.

The radon fluence rates, measured at 24 points of a sampling grid of 100 m x 100 m mesh size, was between 0.25 and 7 $Bq.m^{-2}.s^{-1}$. On June 9th afternoon, the wind was blowing from South-East with a windspeed of 3.9 $m.s^{-1}$ at z = 10 m. The weather was slighlty unstable (L = -30 m). Under these conditions, the radon concentration was measured at 2 m above ground level and 7 differents places on the site. The concentrations, averaged over 1 hour, ranged from 30 to 160 $Bq.m^{-3}$. Three of the values were between 30 and 50 $Bq.m^{-3}$, two between 50 and 100 $Bq.m^{-3}$, and two between 100 and 160 $Bq.m^{-3}$. The values obtained will be compared to those obtained using different models.

2) Modelling of radon dispersion from ground-level sources

The dispersion of radon and more generally of any passive tracer from the soil into the atmosphere has been tentatively described by numeric integration of the Gaussian plume model over the surfaces of interest. Unfortunately, this basic model has been established for elevated sources and for the calculation of ground-level concentrations at relatively large distances from the sources (beyond 100 m). Also, we may have some doubt about the validity of such a model for emission at ground-level, short-range transport and diffusion of the tracer in a thin layer of the atmosphere where gradients of wind speed and diffusivity are relatively important. It would be better to adopt the theoretical profiles classically derived from the diffusion equation and based on realistic profiles of wind speed and eddy diffusivity near the ground. These theoretical profiles are represented by the Robert's equation, reported by Sutton and Pasquill, and reformulated by Van Ulden and Horst:

$$C / Q = a / \bar{u}\, \bar{z}\, \exp [-b (z / \bar{z})^s]$$

where C is the cross-wind integrated concentration at a point situated at a distance x from a point source of emission rate Q. In this formula $\overline{u}$ is the mean horizontal velocity of the tracer molecules, and $\overline{z}$ the mean height of molecules that have travelled a distance x. The constant parameters a, b and s depend on weather stability.

The above concentration distribution is close to an exponential form (s = 1, a = 1, b = 1) for unstable weather and close to a Gaussian form (s = 2, a = 0.64, b = 0.32) for stable weather. In the intermediate situation of neutrality, the constants that ensure the best fit with experimental data would be (s =1.5, a = 0.73, b = 0.54). In the formula, the mean horizontal velocity and the mean height of the diffusing molecules are implicit functions of downwind distance. These parameters can be determined in a relatively simple way by using a calculation scheme that requires only to know or determine the Obukhov lenght, the roughness height, and the windspeed at a given height (Van Ulden 1978).

The air concentration from an uniform area source can be obtained by integrating numerically the formula above over the distance x from upwind edge of the source. If x is large in comparison with the crosswind extent of the source, the formula has to be corrected accordingly. Fig.1 gives an example of calculation of concentration for a radon fluence rate of 1 $Bq.m^{-2}.s^{-1}$ and the meteorological conditions that occurred at St Pierre on June 9th afternoon. This concentration was maximized by assuming that x is small compared with the crosswind extent of the source.

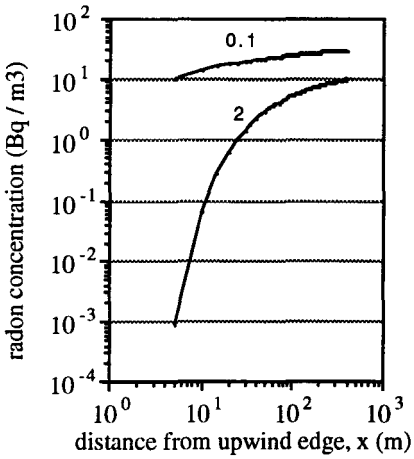


Fig.1: Radon concentration at two heights above ground level (0.1 m and 2 m) calcultated at a point situated downwind an uniform area source (radon fluence rate: 1 $Bq.m^{-2}.s^{-1}$). Meteorological conditions: weather slightly unstable (L = -30 m), moderate windspeed (u = 3.9 $m.s^{-1}$ at z = 10 m) and small roughness height (z_0 = 0.01 m).

Concentration increases from upwind edge of the source to attain a value of about 10 $Bq.m^{-3}$ at x = 400 m for a fluence rate of 1 $Bq.m^{-2}.s^{-1}$ and a sampling height of 2 m. The maximum fluence rate measured at St Pierre being 7 $Bq.m^{-2}.s^{-1}$, the radon concentration at any place should be much lower than 70 $Bq.m^{-3}$. Some of the measured concentrations reach actually much higher values. We are now examining the measurement artefacts or the model deficiencies that could explain the discrepancy. The simple model above could be easily adapted to the determination of long-term average concentrations using wind and stability frequency data.

(Main contributors: M.C Robé, F. Goutelard, H. Camus and S. Raviart)

Head of project 2: Prof. Roehnsch

II. Objectives for the reporting period

The BfS tasks for the joint project are concentrated on: (a) collecting data on site history, nature and amount of residues at the Lengenfeld site; (b) evaluating of available data and acquiring field data from measurements at Lengenfeld site; (c) comparing field data and model predictions.

The objectives for the reporting period were the data collection, carring-out and evaluation of long-therm measurements of Rn-222 in the atmosphere, and the preparation of the experiments in Lengenfeld based on the experience gained by the participation in the St. Pierre investigations.

During the next reporting period the BfS activities will be focussed on the field experiments in Lengenfeld. The BfS will measure metorological data and short-time concentrations of radon and radon progeny in the ambient air.

III. Progress achieved

1. Site characteristics

The Lengenfeld site ist located in a flat valley ascending from southwest to northeast about 400 m above sea level. Surroundings are hilly.

Based on geographical, geological and hydrogeological reports and maps an extended description of the site itself could be made available. Former technical operations at the site - processing of tungsten ores, after World War II of uranium ores, since the early sixties of flourite and baryte minerals - including the use of buildings, facilities and terrain have been explored. Radioactive-contaminated areas have been identified. Origin, kind and distribution of radioactive materials have been clarified and their extent, thickness and radionuclide inventory determined. Thus, the relevant radiation sources at the site can reliably be characterized. Furthermore, ground and surface water has been probed and extended radon measurements at the site and its surroundings performed.

2. Long-term measurements of Rn-222 in the atmosphere at Lengenfeld site

One of the objectives of the atmospheric pathway research is the estimation of dispersion parameters required for longterm modelling at the Lengenfeld site. A validation of this modell may be performed by measurements of long-term radon concentrations.

For the Lengenfeld site favourable conditions exist because of the available data base (e.g. dimensions of tailings pond and piles, waste rock piles and other contaminated areas, concentrations of radionuclides in the materials, local dose rate).

Since two years the BfS is carrying out long-term measurements of Rn-222 in the atmosphere at and in the vicinity of the site. Diffusion chambers are used equipped with solid state nuclear track detectors (MAKROFOL DE 1-4; 300 µm). Detectors exposed are evaluated after electrochemical etching by an image evaluation system consisting of commercial components. The exposition time of the radon detectors is about half a year. The evaluation of the detectors is carried out by the BfS itself. On the former tailings pond and in its surrounding area 15 detectors and in the further distance (up to approx. 10 km) 27 detectors are installed.

The Rn-222 concentrations are influenced by the geological conditions as well as by deposited tailings, i. a. by small areas contaminated with tailings materials due to a former average at the tailings pond. On and very near the source (tailings pond) a range of radon concentrations from 22 to 680 Bq/m^3 (Median: 75 Bq/m^3) was found. The value of 680 Bq/m^3 was measured on the main area source (Lenkteich) directly. The measurements in adjacent sections yield a range from 10 to 85 Bq/m^3 (Median: 30 Bq/m^3). The results of measurements in the further distance of the area sources indicate the prevailing influence of the geology. Thus, it was found that the median of Rn-222 concentrations measured above granite is 33 Bq/m^3 (Kirchberger Granit) respectively 31 Bq/m^3 (Bergener Granit). The median above phyllite is 23 Bq/m^3, the difference being caused by the wellknown variations of Ra-226 (the mother nuclid of Rn-222) concentrations in the rocks.

In the residential area of Lengenfeld situated next to the sources Rn-222 concentrations up to 130 Bq/m^3 have been measured.

3. Preparation of the experiments at Lengenfeld

BfS has carried out the following activities in preparing the short-term investigations in Lengenfeld/Vogtland:

On the basis of the site history data and the available data of local dose rates and radionuclide concentrations the measuring points for the Rn-222 fluxes have been selected. In addition the sampling places have been prepared (clearing the vegetation over a surface area of approximately 1 m^2 and labelling the point by a peg with an assigned number including the geographical coordinates, and registering them in a map).

A second meteorological data acquisition system has been procured and installed. The equipment consists of a 10 m tower with sensors to measure wind direction, wind speed and temperature. The data are recorded with one-minute intervals on a data logger.

Besides, extensive organizational, logistical and technical activities have been initiated and carried out in order to pepare the joint IPSN-GRS-BfS experiments at the Lengenfeld site appropriately, scheduled for 12 - 22 September.

Head of project 3: Dr. Maßmeyer

ii. Objectives for the reporting period

GRS will concentrate on modelling the atmospheric dispersion of radon and radon progeny from large area sources with models of different complexity. Data obtained by IPSN and BfS on a German (Lengenfeld, Saxony) and French (St. Pierre du Cantal) site will be used to drive and validate the models.

In order to predict population exposures in complex terrain, a detailed modelling of flow and turbulence fields in the atmophere is necessary. Hence, coupled flow and dispersion models will be implemented to handle large area sources enabling the calculation of atmospheric dispersion under the influence of inhomogeneous topography.

The atmospheric models will be driven and validated based on the measurements of IPSN and BfS. The difference between the results of a simple Gaussian type model and those of advanced modelling techniques described above will be evaluated.

The measurements include the determination of the meteorological input parameters for dispersion modelling as well as mapping of radon fluxes and air concentration data around the disposal sites during short periods of time (~ 1 h). Concerning the experimental setup, a two step procedure has been envisaged: First - in order to check the turbulence parameterization and source modelling approach - the ability of the models to handle single area sources will be investigated. In a later stage and depending on special characteristics of source distribution in Lengenfeld combined effects of multiple area sources in uneven terrain will be studied.

During the reporting period the experimental areas have been identified. Based on site inspections the experimental setup has been finally designed. The equipment of a meteorological data acquisition system (tower measurements) - supplied by BfS - has been discussed. A screening concernig the availability of the necessary input parameters for the dispersion models has been carried out.

In addition meteorological data measured on an hourly basis over a period of nearly half a year at the site of Lengenfeld have been transferred from BfS to GRS. These data should be used to calculate long term dispersion factors at the site of Lengenfeld using a simple Gaussian type model. The adaption of flow and dispersion models has been started.

Objectives for the next reporting period:

During a period of one or two weeks in September a field experiment will be carried out in Lengenfeld to measure horizontal variability and time dependence of radon exhalation, radon concentration in the ambient air and meteorological conditions. A simple Gaussian type model and more advanced wind field and dispersion models will be applied and the results will be compared with the experimental data thus allowing a validation of the models. To describe long term radon concentration in the environment near large area sources, meteorological data and radon concentration data sampled over a half year period in the region of Lengenfeld will be used for validation of a simple dispersion model (MILDOS-code).

iii. Progress achieved including publications

The work carried out during the reporting period is related to the topics

- adaption of a simple model
- preparation of a model for complex terrain
- preparation of validation experiments

1. Adaption of a simple model:

As a simple dispersion model to describe atmospheric dispersion from area sources the updated version of the MILDOS-AREA code (1989) supplied from the NEA data bank has been implemented at GRS. This code is designed for execution on personal computers. It can handle emissions of radioactive material from fixed point source locations and from area sources. Those sources are modelled using a sector averaged Gaussian plume dispersion model which utilizes user provided wind frequency data. Mechanisms such as deposition of particulates, resuspension, radiactive decay and ingrowth of daughter radionuclides are included in the transort model. Concerning the dispersion modelling the major revision from the original MILDOS code (1981) concerns

- the treatment of atmospheric dispersion from area sources via a finite-element integration approach (the original code uses the virtual piont source method) and
- the implementation of Martin-Tickvart dispersion coefficients (adapted to surface releases) instead of Briggs coefficients (adapted to elevated releases).

In the present version of the model annual average air concentrations are computed.

Because it is a simple Gaussian type model based on meteorological measurements at one location, MILDOS is not able to describe the influence of uneven topography (and the accompanying inhomogeneous wind field) on the process of atmospheric dispersion. It should be used as a reference model for area sources in level terrain.

Since September 1991 radon concentrations have been mapped at different locations in the vicinity of the area sources in Lengenfeld. Simultaneoulsly meteorological data (small tower) on an hourly basis have been measured at a single location during more than half a year. All measured data are available and will be evaluated in comparison to the MILDOS results within a thesis at the Fachhochschule Aachen from October 1993 to February 1994. As already mentioned above the MILDOS-code supposes area sources to be situated in flat terrain. It is expected that - due to inhomogeneous wind fields - the differences between calculated and measured data will become more pronounced as the distance between radon monitors and the meteorological tower grows.

It is planned to modify the dispersion part of MILDOS enabling the calculation of short dispersion periods. Based on this MILDOS version a comparison with other enhanced dispersion models (Gaussian-Puff-model, Lagrangean particle model) will be carried out. The calculational results will be compared with experimental data from Lengenfeld. This allows to assess the performance of the different modelling approaches.

2. Preparation of a model for complex terrain:

As stated above (compared to MILDOS) a more realistic approach to model atmospheric dispersion of area sources in uneven terrain is based on mesoscale flow models coupled with appropriate dispersion models. GRS will apply a hydrostatic flow model to calculate the large scale features of the flow field in the area of Lengenfeld. These results will be used to drive a smaller scale diagnostic flow model producing a mass consistent flow field under the influence of topography. These wind field data and associated turbulence information form the input for different dispersion models (Gaussian puff, Lagrangean and Eulerian type models). The results of these models will be compared with measured concentration data. To initialize the model runs the following datasets must be available:

- for the calculation of atmospheric flow field
  - digitized orographical data concerning height above sea level in a specified grid
  - digitized land use data
  - wind data driving the flow model, e.g. geostrophic wind speed and direction at sea level and 850 hPa level
  - initial temperature profile (nearest radiosonde ascent and near surface temperature)
- for the calculation of atmospheric dispersion
  - the wind and turbulence data at the grid points (results of flow calculation)
  - source conditions (position, area, source strength, characterization of the radionuclides)

Test calculations with the coupled model system will be started in the beginning of October. These calculations will be based on digitized orographical data in an area of 40km x 40 km centered around Lengenfeld. The horizontal grid size is 250m.

3. Preparation of validation experiments:

To become familiar with the measurement technique of IPSN a pilot experiment has been carried out in St. Pierre du Cantal which has been accompanied by GRS/BfS. During this experiment the performance of the French measuring devices has been demonstrated and of the necessary preparational work at the site of Lengenfeld has been discussed.

The site of Lengenfeld has already been inspected. The locations of long term measurements of radon concentration in ambient air - carried out since 1991 - have been identified. Logistic problems concerning the field experiment in September have been discussed and potential locations for the measurement of meteorological data as well as source term and radon concentration in the environment have been specified.

During an additional site inspection in Lengenfeld in the beginning of September the positioning of measuring devices depending on wind direction will be determined and the final experimental design will be proposed. The experimental setup of a meteorological data acquisition system - which has been purchased by BfS for supplementary meteorological data - has been agreed upon and possible locations for the installation have already been identified.

Progress Report

Contract: **FI3P-CT920010** **Sector: A23**

Title: The bio-availability of long-lived radionuclides in relation to their physico-chemical form in soil systems.

1)	Lembrechts	RIVM
2)	Wilkins	NRPB
3)	Cremers	Univ. Leuven (KUL)
4)	Merckx	Univ. Leuven (KUL)
5)	Staunton	INRA
6)	Berthelin	CNRS
7)	Mocanu	Inst. Atomic Phys./IFIN

I. Summary of Project Global Objectives and Achievements

For a number of radionuclides, $^{134}Cs/^{137}Cs$ and ^{90}Sr being the most important ones, the variables which primarily define the interactions with the main soil constituents and the accumulation by plants and soil (micro)organisms are investigated. The aspects which influence solid/liquid distribution and the aspects which affect uptake and accumulation are studied separately and in combination, under controlled conditions.

Objectives of the proposal are:

- Study the uptake of nuclides from the soil liquid by different organisms, in relation to major characteristics of the soil.
- Study the efficiency of the uptake process (properties of the organism affecting uptake).
- Evaluate the effects of common soil treatments on solid / liquid equilibria in soils in order to select the most appropriate corrective measure to use following contamination of agricultural land with radiocaesium and radiostrontium.
- Develop rapid methods to predict the effect of measures in the event of an accident.
- Assessment of the value of fractionation techniques meant to quantify the available fraction by comparing obtained results with uptake patterns.
- Describe the role of soil μ-organisms in the leaching and retention of caesium in soils.
- Develop a dynamic mechanistic model to simulate the time dependent soil/solution/root distribution of Cs; sensitivity analysis as a tool for efficient experimental design.

Selection of activities and achievements:

- Two contractors meetings were held, the notes and technical information of which has been sent to the CEC scientific official.
- Some of the groups that recently got involved in the project focused on the preparation of their experimental programme and an introductory bibliographic study. A fist contractor from Eastern Europe joined the group.
- A number of soils and soil materials used in this project were extensively characterised, their potassium release characteristics and the dependence of Cs-fixation on time were determined, and the effects of Cs-concentration and ionic strength on the Kd of radiocesium were examined, the non-linearity of these materials being confirmed.
- The effect of changes in level of nutrients on the TF of radiocaesium to spinach was comparable to effects which have been observed for lettuce. The effect of ionic composition on the transfer factor was tentatively attributed to the loading of the nuclide in the root exchange complex at the plasmalemma.
- The accumulation of radiocaesium by earthworms was shown to be species dependent, not affected by pH and to be modified in the presence of potassium or stable cesium.

Head of the project 1: Dr. Lembrechts

IIa. Objectives for the reporting period

1. Study the transfer of ^{134}Cs along the soil-soil solution-organism interfaces under different environmental conditions. At first experiments were executed on the equilibrium between solution and organism.
2. Study of the kinetics of ^{134}Cs uptake in different earthworm species in solution to describe 1. the speed of uptake and release and 2. the relative importance on intra species differences.
3. Execute experiments with earthworms in solution in which key factors, such as pH, calcium concentration, potassium concentration are varied, to determine their influence on cesium-134 concentration in organisms.

IIb. Objectives for the next period

1. Study the transfer of ^{134}Cs along the soil-soil solution-organism interfaces by executing experiments in solution and in soil under different environmental conditions. Experiments in solution will be finished in autumn 1993, experiments in soil have been started in recently and will be continued in the next period.
2. Describe the kinetics of ^{134}Cs uptake in different earthworm species in soil. The results will be compared with the results from our experiments in solution and will give information on the bioavailable fraction.
3. Execute experiments with earthworms in soil in which key factors, such as pH, calcium concentration, potassium concentration are varied to determine their influence on cesium-134 concentrations in organisms.
4. Comparison with the results from the experiments in solution will provide information on the relative importance of processes at the soil-soil solution interface and those at the soil solution- organsism interface for uptake of ^{134}Cs.
5. Evaluate the effects of different environmental parameters on the process of uptake of cesium-134 by earthworms and identification of main sites of action; soil - soil solution interface or soil solution - organism interface.

III. Progress achieved including publications

1. Preliminary experiment

1.1. Materials & methods.
Three earthworm species *Lumbricus rubellus*, *Eisenia foetida* and *E. andrei* were kept individually in 25 ml of artificial solution with 5 Bq/ml (Cs-134) for two weeks at 15 °C (light/dark 12/12). The solution has also been used by other investigators studying the kinetics of chlorobenzenes in earthworms (Belfroid *et al.*, 1993). During two weeks individuals were sampled at different time intervals and activity was measured. Results were mainly used for planning of the other experiments.

1.2. Observations.
Uptake kinetics of ^{134}Cs in the earthworms *E. foetida* and *E. andrei* looked similar, cesium was taken up much slower by *L. rubellus*. It seemed that in both *Eisenia* species an equilibrium was reached after about 100 hours, the concentration factor being about 20.

The equilibrium was not reached in *L. rubellus*. The concentration factor after 400 hours was about 7.

2. Uptake kinetics.

2.1. Materials & methods

Two earthworm species *E. foetida* and *L. rubellus* were kept individually in solution for two weeks. Six individuals from each species were sampled at different time intervals and activity was measured. Laboratory conditions were similar to the first experiment except for the amount of activity in solution (50 Bq ^{134}Cs/ml). The uptake and release process was described by parameters which were calculated using a one compartment model described by the formulae $C_t = A/k.(1-e^{-kt})$ in which C_t is the concentration at t=t in Bq/g d.w., A is assimilation in Bq/g/hr, k = excretion constant in hours^{-1} and t is time in hours.

2.2. Observations.

The kinetics of cesium-134 showed to be quite different in both species. Both uptake and elimination showed to be higher in *E. foetida* than in *L. rubellus*. The equilibrium concentration, which can be calculated from both the uptake (A) and elimination parameter (k) through the formula equilibrium concentation = A/k, was twice as high in *E. foetida*. Concentration factors from soil solution to earthworm, calculated from the equilibrium concentrations, were 37 and 18 for *E. foetida* and *L. rubellus* respectively. Biological half life were 58 and 231 hours respectively. The results show that equilibrium of ^{134}Cs in earthworms is reached quite rapidly compared to some other elements. Results are summarized in table 1, the uptake process is shown in fig. 1.

Table 1. Uptake and release parameters of ^{134}Cs kinetics in two earthworm species.

	A (Bq/g/hr)	k (hrs^{-1})	activity at equilibrium (Bq/g dw)	T½ (hrs)
E. foetida	22	0.012	1800	58
L. rubellus	2.7	0.003	900	230

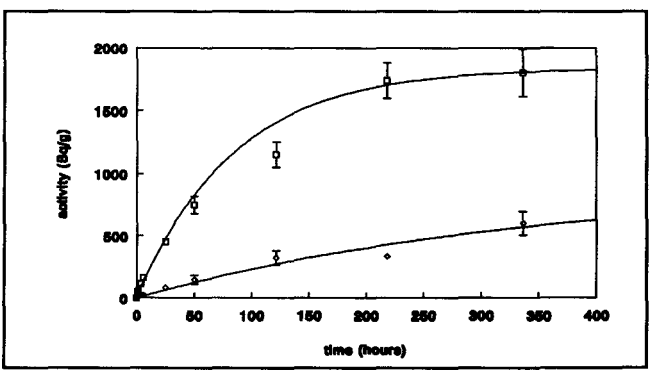


Fig. 1. Uptake of ^{134}Cs by the earthworms *E. foetida* and *L. rubellus*.

3. Effect of potassium and caesium on the uptake of ^{134}Cs by *E. foetida*.

3.1. Materials & methods

Earthworms (*E. foetida*) were kept at 15 °C in a solution with 50 Bq ^{134}Cs/ml. The experiments consisted of six different treatments, 3 potassium concentrations, 0, 1 and 10 mmol/L and 2 of stable caesium. concentrations, 0 and 0.0075 mmol/L. The concentration ranges of both elements reflect those observed in solution. Individuals were sampled after two weeks.

3.2. Observations

It was expected that both potassium and caesium might compete for binding sites at the soil solution-earthworm interface and thus reduce uptake of the radionuclide. Addition of potassium and caesium in the solution lowered the activity in the earthworm significantly, no interaction between the two elements was observed. The effect of potassium was comparable to the effect of caesium when expressed per mol added. However, as the concentration range of potassium added was much larger, a larger total effect was observed. It is obvious from the results that the effect decreases with increasing potassium concentration. Similar results were obtained in an experiment with *L. rubellus*. The results for *E. foetida* are summarized in fig. 2.

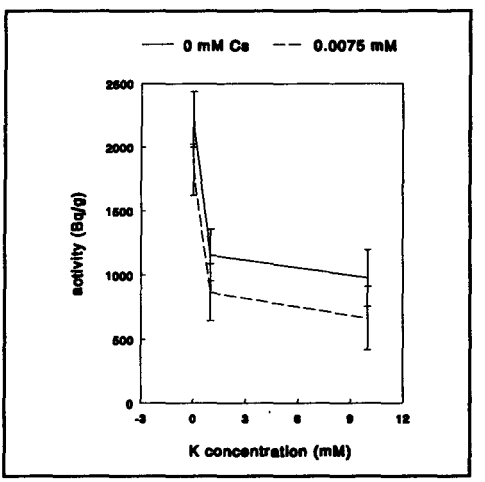


Fig. 2. The effect of K and Cs on the uptake of ^{134}Cs by earthworms in solution.

4. Effect of pH on the uptake of ^{134}Cs.

4.1. Materials and methods

Before starting the pH experiment a preliminary experiment was executed in which shift in pH was studied in solution with and without earthworms. In all cases pH changed progressively, depending on the original pH, on the presence of earthworms and depending on the species. Absolute changes were largest in the first 5 hours. It was decided to check pH in the course of the experiment and to refresh the solution when the shift was larger than 0.5.

The set up of the experiment was identical to the previous one, except for the amount of

liquid per pot which was enlarged to 100 ml. pH's were set to 4, 6 and 8 by adding HCl or NaOH. Shifts in pH were biggest in the treatments pH 4 and 6, those were refreshed every 100 hour, no refreshment of medium was executed in the pH 8 experiment. Samples were collected after two weeks.

4.2. Observations

It was hypothesized that the effect of pH would be mainly on the soil-soil solution interface, relatively small effect was expected on the soil solution-earthworm equilibrium due to effects on the physiology of the worms.

As already stated above earthworms were able to cause a considerable shift in pH, especially in the lower pH ranges. If such an effect occurs under field conditions this may cause a considerable effect on availability by affecting the equilibrium at the soil-soil solution interface, thus changing the solubility. In our experiments no significant effect of pH on the ^{134}Cs uptake by *E. foetida* or *L. rubellus* from solution was observed although uptake was increased a little in *E. foetida* at pH 6. The difference between the two species however was significant at all pH's. No interaction between pH and species was observed. The results are given in fig. 3.

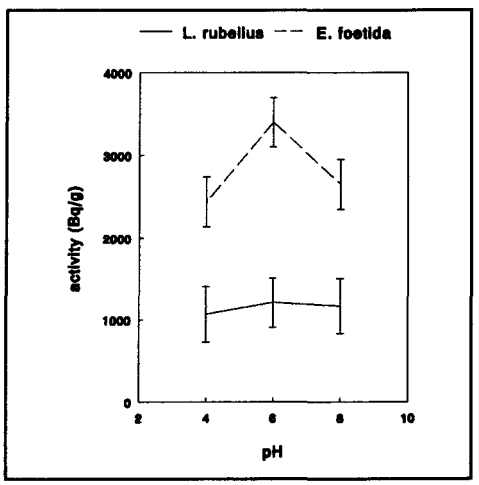


Fig. 3. The effect of pH on the uptake of ^{134}Cs.

5. Further experiments in solution

5.1. Materials and methods

Experiments on the effect of potassium and cesium on ^{134}Cs uptake in Lumbricus rubellus and on the effect of temperature on the ^{134}Cs uptake in both species have already been executed under comparable laboratory circumstances as above. Measurements and calculations will be finished in september 1993. A Last experiment on the effect of ammonia and calcium will be executed in september/october 1993.

Publications

M.P.M. Janssen, P. Glastra & J.F.M.M. Lembrechts. 1993. Waardoor wordt de opname van cesium-134 door regenwormen bepaald? Symposium Netherlands Integrated Soil Research Programme, Lunteren, The Netherlands.

Head of project 2 : Drs Wilkins & Nisbet

IIa Objectives for reporting period

(a) To characterise fully five diverse soil types selected for study with regard to their mineralogy, organic matter and nutrient status;
(b) To develop a methodology to determine the potassium release characteristics of various soils so that optimum potassium treatment rates can be set for different soil types following contamination with radiocaesium;
(c) To conduct the first set of batch equilibrium experiments on five diverse soil types six months after contamination to determine which common soil treatments may be applied after an accident to maintain Cs:K and Sr:Ca quotients in solution as low as possible.

IIb Objectives for next reporting period

(a) To complete analyses from the first set of batch eqilibrium experiments;
(b) To conduct a further set of batch equilibrium experiments on the five soils one year after contamination;
(c) To expand the present data base on potassium release characteristics of the five soils by more extensive Quantity-Intensity studies;
(d) To make recommendations on treatments, treatment combinations and treatment rates which may be applied to soils after an accident to reduce the availability of radiocaesium and radiostrontium to plants;
(e) Prepare final report.

III Progress achieved including publications

(a) Soil selection, sampling and characterisation

The five diverse soil types selected for study comprised three agricultural soils (Hamble loam, Fyfield sand and Adventurers peat) which have already been used in previous NRPB investigations of soil-to-plant transfer (1,2), and two upland organic soils (deep peat and peat ranker) from established NRPB field sites. Twelve porous ceramic cups were installed at each sampling site at the start of this investigation to collect soil solution for subsequent use in batch equilibrium experiments. Soils were sampled at 0-15 cm depth adjacent to the porous cups, and characterised in terms of mineralogy, organic matter and nutrient status. The soils were shown to be of a diverse nature (Table 1) in all aspects except for the % clay content and types of clay minerals present.

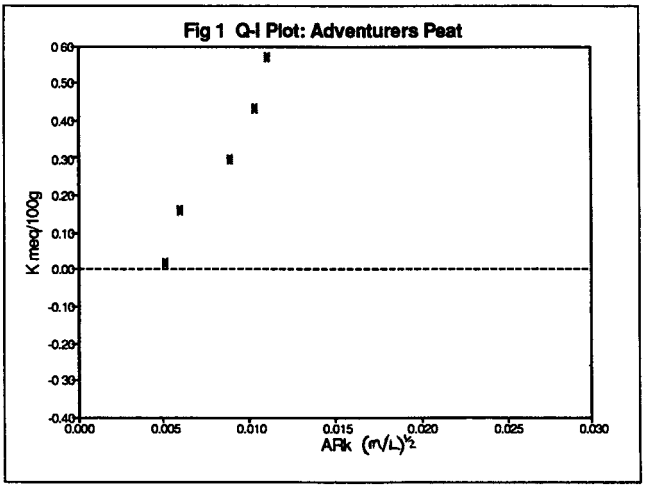
(b) Potassium supply in soil

In order for potassium to be used effectively as a countermeasure for reducing radiocaesium uptake by plants, more information on the ability of different soil types to supply potassium is required. Potassium supply is characterised by both the labile amount of potassium in soil (Quantity) and its concentration in soil solution (Intensity). The ability of the solid phase to replenish the potassium ion in solution when it becomes depleted depends on the buffer capacity of the soil. Buffer capacities of the loam,sand and three peat soils were investiagated quantitatively using an experimental technique pioneered by Beckett (1964). Representative

Table 1 Physical and chemical characteristics of the experimental soils

	LOAM	SAND	PEAT (A)[1]	PEAT(PR)[2]	PEAT (DP)[3]
pH ($CaCl_2$)	6.7	6.8	4.7	3.5	3.9
OM (%)	8	2	60	77	82
CEC[4]	21.4	11.3	83.5	100	105.6
Ex K[4]	1.9	0.6	0.4	0.7	0.6
Ex Ca[4]	9.6	3.4	7.5	0.5	3.1
C'rseSand[5]	24	60	5	1	2
Fine Sand[5]	39	18	6	1	2
Silt[5]	18	11	15	10	6
Clay[5]	11	9	14	11	8
Illite[6]	5	3	6	6	3
Expand.[6]	4	5	4	3	4
Kaolinite[6]	2	1	-	2	1
Chlorite[6]	-	-	4	-	-

[1] Adventurers peat
[2] peat ranker
[3] deep peat
[4] meq $100g^{-1}$ dry weight
[5] percent of whole soil
[6] types of clay mineral comprising clay fraction expressed as a percent of whole soil



samples of the five soils were air dried and five 5 g (< 2 mm) subsamples were equilibrated with a series of five solutions containing variable amounts of KCl in $CaCl_2$. For each suspension the difference between the K concentration in the initial and final solution gave the amount by which the soil gained or lost potassium in reaching equilibrium (i.e. the Quantity factor or $\pm \Delta K$). The activity ratio or intensity of K ($AR_K = a_K/(a_{Ca} + a_{Mg})^{½}$) was calculated from the composition of the resulting solutions. A characteristic Q-I curve for Adventurers peat is shown in Fig 1. The slope of the linear portion of the Q-I plot represents the potential buffer capacity (PBC_K) of the soil. Soils with a steep slope i.e. high buffer capacity have a greater ability to sustain potassium supply than those soils with low buffer capacity. Preliminary results from the first set of Q-I experiments on the five soils showed PBC_K for Hamble loam, Adventurers peat, deep peat and peat ranker to be around 73, 70, 42 and 33 meq $100g^{-1}/(mol\ l^{-1})^{½}$, respectively. Insufficient data were available from the sand to make an estimate of PBC_K. In order to produce an accurate assessment of PBC_K for all five soils, the Q-I procedure will have to be repeated with around 20 subsamples of soil per soil type.

(c) Batch equilibrium studies

A previous investigation (3) evaluated a simple batch equilibrium method for use after an accident to predict the effect that different soil treatments may have on radionuclide availability to plants. The study replicated in situ conditions by using field-moist soils and their associated soil solutions. The current set of batch equilibrium experiments extend those conducted previously by:

(i) using soils freshly contaminated with ^{134}Cs and ^{90}Sr (March 1993);

(ii) ensuring that the activity concentrations of ^{134}Cs and ^{90}Sr in these soils were sufficiently high to allow aliquots of the liquid phase to be taken, thereby enabling the kinetics of the exchange process to be followed more closely;

(iii) using soils not previously irrigated with water containing high levels of calcium.

40 kg of soil (0-15 cm depth) from each of the five field sites were collected and transported to NRPB in November 1992. They were allowed to dry at ambient temperature until they were in friable form. In March 1993 each of the five soils were contaminated with ^{134}Cs and ^{90}Sr. Activity concentrations of ^{134}Cs in these soils range from 140-960 kBq kg^{-1} dry wt. Specific activities of ^{90}Sr are currently being determined. The homogeneity of the contamination was checked from measurements of ^{134}Cs in 10 subsamples from each soil. To provide the liquid phase for the batch equilibrium studies, about thirty litres of soil solution were collected from each of the five sites over a period of six months, and stored in a refrigerator.

The first set of batch equilibrium experiments with potassium fertiliser is underway. One control and three treatment rates are being investigated i.e. standard (200 kg ha^{-1}), five times standard and ten times standard agricultural application rates. Field moist soil samples and their associated soil solutions were shaken with the appropriate treatment and five aliquots taken during the 48 hour contact period. The distribution of ^{134}Cs, ^{90}Sr, K and Ca in the liquid phase is being determined and quotients of ^{134}Cs:K and ^{90}Sr:Ca calculated. Any treatment found to decrease these quotients compared to controls is being considered as a potentialy effective countermeasure for reducing uptake of the appropriate radionuclide by plants. Preliminary results indicate that equilibrium is reached very soon after contact with the

potassium treatment. Data collected so far support findings of a previous study (3) in which potassium was shown to be a particularly effective countermeasure in peaty soils.

(d) Publications issued during reporting period

1 Nisbet A.F. and Shaw, S. (in press) Summary of a 5 year lysimeter study on the time-dependent transfer of ^{137}Cs, ^{90}Sr, $^{239,240}Pu$ and ^{241}Am to crops from three contrasting soil types: 1 Transfer to the edible portion. J. Environ. Radioactivity.

2 Nisbet, A.F. and Shaw, S. (in press) Summary of a 5 year lysimeter study on the time-dependent transfer of ^{137}Cs, ^{90}Sr, $^{239,240}Pu$ and ^{241}Am to crops from three contrasting soil types: 2 Distribution between different plant parts J. Environ. Radioactivity.

3 Nisbet, A.F., Mocanu, N and Shaw, S. (in press) Laboratory investigation into the potential effectiveness of soil-based countermeasures for soils contaminated with radiocaesium and radiostrontium. Sci. Tot. Environ.

References

Beckett, P.H.T. (1964) Studies on soil potassium 1 Confirmation of the ratio law: measurement of potassium potential. J Soil Sci., 15, 1-8

Head of project 3: Prof. Cremers

II. Objectives for the reporting period

1. Nutrient solution composition and TF's in plants
2. Solid phase speciation of radiocaesium in soils
3. Fixation dynamics of radiocaesium in organic matter soils
4. Radiostrontium sorption-desorption behaviour in soils

III. Progress achieved including publications

1. Nutrient solution composition and transfer factors for radiocaesium

In collaboration with R. Merckx, Laboratory of Soil Biology and Fertility, a comprehensive study was carried out on the effect of nutrient solution composition on the uptake of radiocaesium in spinach plants. The concentration of the major cations Ca, Mg and K was varied over a wide range and a number of protocols was set up in which ammonium was included. In total, 15 scenarios were studied covering total concentrations in the range of 5 to 13 meq/L. Absence of nutritional stress was evidenced by identical values (within about 7%) of relative growth rate (0.4 day^{-1}) in the exponential growth phase. TF values covered a range of 40 to 120 L/Kg (ovendry).

It was demonstrated that K and NH_4 ions can be interchanged (keeping K at some reasonable level) at otherwise identical Ca+Mg concentrations without any effect whatsoever on TF values. No

correlation could be demonstrated between TF and single ion concentrations.

However, a quantitative aproach was developed allowing to rationalize radiocaesium TF on the basis of overall cationic composition (Ca,Mg,K,NH_4) of the nutrient medium. The starting point in the approach is that radiocaesium taken up in the plant is first order with respect to the radionuclide loading in the root exchange complex at the plasmalemma. Prior to uptake, the nuclide must pass through the cell wall free space characterized by some specific cationic exchange capacity, the bulk fraction of which is associated with COOH groups. Consequently, it can be expected that the radiocaesium loading shall be influenced by the ionic composition of the ion exchange complex which in turn is influenced by the composition of the nutrient medium.

In calculating the ionic composition of the root ion exchange complex, we make two assumptions. Firstly, we take a unit ion exchange selectivity coefficient for the K/NH_4 and the Cs/K pairs. The interchangeability of K and NH_4 without any effect on TF, as discussed above justifies such an assumption. It may be further justified on the basis of findings showing very small differences in selectivity among poorly hydrated cations in COOH-bearing materials such as humic acids. Furthermore, on the basis of literature data it appears that, even for the K-Na pair, the calculated selectivity coefficient is not larger than 2 in the root exchange complex. We take a similar assumption for the Ca/Mg pair. From literature data, it can be calculated that the Ca/Mg selectivity coefficient is about 1.5. Since in most scenarios calcium concentrations are more than twice those for magnesium, we expect that this approximation will have limited impact on the calculations. Consequently, in the following, we shall treat K and NH_4 as <u>one</u> ion, representing it by M^+ and do the same for Ca+Mg, represented by M^{++}. On the basis of a unit selectivity coefficient for the Cs^+/M^+ pair, we may write

$$[Cs]_{plant} = k \frac{(M^+)}{[M^+]} [Cs]_{solution} \qquad (1)$$

in which (M^+) refers to the fractional loading of M^+ in the root ion exchange complex and $[M^+]$ the molar concentration of M^+ in the nutrient medium. k is a proportionality constant between uptake and radiocaesium level in the exchange complex, reflecting the efficiency of the transport process across the plasmalemma. TF may thus be written as

$$TF = k \frac{(M^+)}{[M^+]} \qquad (2)$$

The message conveyed by eqn (2) is that TF is linearly related to the size of the "potassium window" in the cortical apoplasm and inversely proportional to the level of the M^+ competitive ion in solution. If we were to explicit (M^+) in absolute terms (by multiplying with the CEC of the plant root) then eqn(2) simply states the proportionality between TF and the root/solution partition

coefficient of M^+ (and of course radiocaesium) making k a dimensionless constant.

The remaining task is to express (M^+) in terms of nutrient solution composition. This can be done in classical ion exchange terms on the basis of a selectivity coefficient of the bivalent cations M^{++} (Ca and Mg) towards the monovalent ions (K and NH_4).

Experimental data have been fitted to eqn(2), using $K_c(M^{++}/M^+)$ as an adjustable parameter. The result is shown in Figure 1. It is apparent that experimental behaviour is perfectly well described by eqn.(2), the linear regression being characterized by a value of $R^2 = 0.98$ for this no-intercept model. The best-fit value for $K_c(M^{++}/M^+)$ is 0.27 mol/L, a value which is consistent with known selectivity behaviour in COOH-bearing materials.

The excellent agreement allows to make some general predictions on the nature of the specific ionic effects in hydroponic uptake studies. Increasing the M^+ concentration (keeping M^{++} constant) displaces radiocaesium towards the nutrient solution (a linear term) but this effect is partly offset by a (non-linear) increase in the size of the "potassium window" of the root exchange complex (M^+). An increase in $[M^{++}]$ concentration, keeping $[M^+]$ constant, leads to a decrease in (M^+) (non-linear) resulting in a direct effect of TF.

However the field scenario is quite different in the sense that $[M^+]$ cannot be changed without changing $[M^{++}]$ and vice versa. In fact, it is readily seen that, since the amount of fertilizer added is only a small fraction of the total ionic pool in the soil exchange complex, the ratio of $[M^+]^2/[M^{++}]$ in the soil solution is unchanged since it is buffered by the composition ratio $(M^{++})/M^+)2$ in the soil exchange complex. The cationic composition of the root ion exchange complex is unchanged and consequently, the net effect of either liming or K-fertilization is an increase in total ionic concentration and an increase in K concentration, leading to a decrease in TF. The beneficial effect of calcium is therefore indirect through a raise of the K-level in the soil solution.

2. Solid phase speciation of radiocaesium in soils

The behaviour dynamics of radiocaesium in soils is directly connected with its speciation in the solid phase. In the past a number of methods have been developed for quantitatively characterizing soils in terms of specific sorption properties (illitic frayed edge sites, FES) and specific radiocaesium interception potentials. These methods were based on the use of masking techniques for the regular exchange complex (REC).

New methods have now been developed to demonstrate directly the involvement of specific sites in the radiocaesium sorption in soils. These methods are based on the measurement of the response of radiocaesium sorption coefficients to the addition of ammonium in the absence of masking agents. The experimental scenario is as follows. Soils are preequilibrated with a Ca-K solution (Ca=100mM; K=10mM)

containing varying levels of NH_4 (up to 5mM). Potassium adsorption ratios, defined as m_K/ m_{Ca} are 1 and represent upper limits of values in soils, thus corresponding to upper limits of K saturation in the regular exchange complex and therefore of the possible involvement of this complex in radiocaesium sorption. $K_D(Cs)$ is measured for all scenarios.

Equations were developed for predicting S/L partitioning. If radiocaesium is exclusively confined to the FES, we obtain:

$$\frac{(K_D.m_K)}{K_D.m_K} = 1+K_c(N/K)\ m_N/m_K \qquad (3)$$

in which $(K_D.m_K)$ refers to the $K_D^{Cs}.m_K$ product in the Ca-K scenario, $K_D.m_K$ to that product in the Ca-K-NH_4 scenario, $K_c(N/K)$ the NH_4-to-K selectivity coefficient in the FES and m_N/m_K the ratio of concentrations in the liquid phase. For a mixed behaviour, involving the action of both FES and REC, we obtain

$$\frac{(K_D.m_K)}{K_D.m_K} = \frac{1 + R}{(1+K_c m_N/m_K)^{-1} + R} \qquad (4)$$

in which R refers to the ratio of interception potentials of REC and FES. When the contribution of the FES vanishes, the $(K_D.m_K)/K_D m_N$ remains unity and is independent of m_N/m_K.

Figure 2 shows the results for illite clay and a set of soils of varying organic matter (OM) content. It is seen that except for soils of very high organic matter the slope is in the range of about 5 to 7, showing directly the involvement of the FES. In the case of OM contents of 97% a zero slope is obtained, demonstrating a unit selectivity coefficient for NH_4/K in the REC and a quantitative interception of radiocaesium in the REC. The peat soil with 88% OM shows a mixed behaviour, describable by eqn.4, whereas for the peat soil with 84% OM, radiocaesium is nearly quantitatively present in FES.

3. Fixation dynamics of radiocaesium in organic matter soils

A comprehensive study was carried out on the fixation dynamics and aging processes in soils characterized by a broad range of organic matter. In some cases, significant aging processes could be demonstrated whereas in other cases significant fixation levels could be demonstrated within a very short time interval after contamination after which time no significant changes occur. The most disturbing observation is that widely differing desorption yields are obtained for soils which are otherwise characterized by similar radiocaesium properties. At this stage, the nature of this erratic behaviour is not understood and we lack the quantitative diagnostic criteria for predicting radiocaesium fixation behaviour on the basis of readily measurable properties.

A similar erratic behaviour is found for the fixation response to calcium additions. In some cases,significant enhancement of

caesium is observed whereas in other cases, there are no measurable effects. It is suggested that structural differences in the FES may be at the origin of this unpredictable behaviour.

4. Radiostrontium sorption-desorption behaviour in soils

A comprehensive study was carried out on the sorption-desorption behaviour of radiostrontium in soils containing a wide range (17-97%) of organic matter.

The sorption of a major fraction of radiostrontium added in soluble form is ruled by simple and reversible ion exchange reactions, involving regular exchange sites on organic matter and clay minerals. In clay minerals, Sr^{2+} is held more preferentially than Ca^{2+}, whilst in humic acids Ca^{2+} is held more preferentially than Sr^{2+}. Ion exchange selectivity differences among Sr^{2+} and Ca^{2+} are rather small and the distribution coefficient of radiostrontium added in trace quantities is a close reflection of the distribution coefficient of the competitive Ca^{2+} ion. However, a small fraction of radiostrontium added in soluble forms becomes fixed in a non-exchangeable form, most probably by strontium-organic matter-clay complexes. This "specific" strontium adsorption may account for the apparent higher Sr^{2+}-to-Ca^{2+} preference as observed in organic soils.

5. Publications

- At this stage, two doctoral dissertations (E.Valcke and L.Sweeck) on the subject of radionuclide behaviour in soils are in the final stage. Public defense of these theses is scheduled within 1993.

- A comprehensive list of publications on the subject of radiocaesium and radiostrontium in soils and sediments (covering the various developments which have taken place in this laboratory in recent years) is now in preparation.

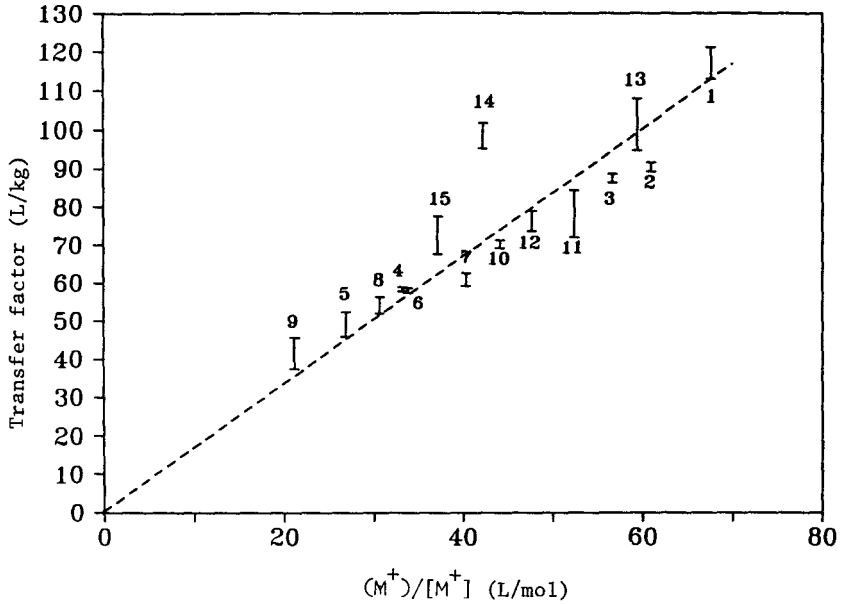


Figure 1. Correlation (R^2= 0.98) between ^{137}Cs transfer factors for spinach plants and the ratio of the fractional monovalent ion loading ($K+NH_4$) in the root exchange complex and the monovalent ion concentration in the nutrient solution.

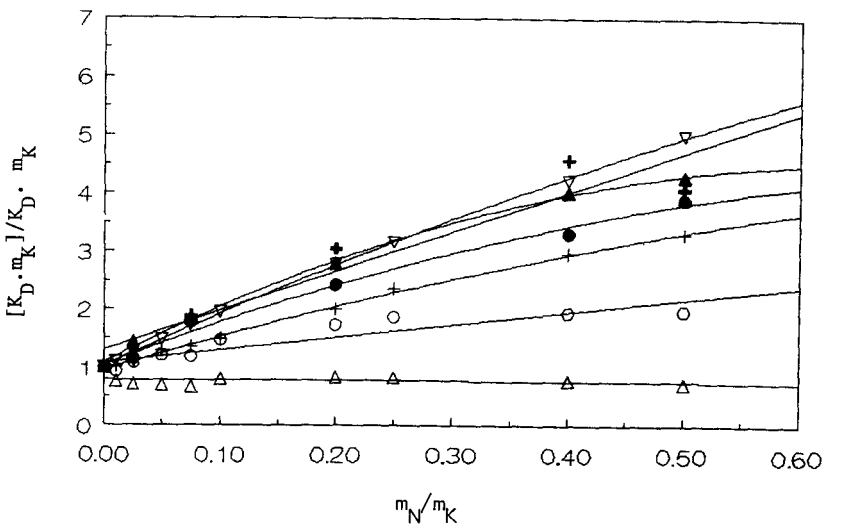


Figure 2. Dependence of radiocaesium distribution coefficients on ammonium concentration (eqn.2)
▽: illite clay, +:acid brown earth (21%OM), ▲:peat soil (84%OM), ●:forest soil (11%OM), +:podzol, o: peat ranker (88%OM), △: peat soil (97%OM).

Head of project 4 : Prof. Dr. Merckx

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Introduction

Radiocaesium contaminated large agricultural areas in Europe after the Chernobyl accident in 1986. Among various agronomic practices, selection of appropriate crops has been demonstrated to be a succesful countermeasure (Alekshakin, 1993). Recently, we proposed hypotheses to explain this genotypic variation in radiocaesium uptake from soil (Smolders and Merckx, 1993). They are: (i) the Cs/K discrimination (the Cs/K observed ratio as defined by Comar, (1956)) is equal for different plants grown at identical conditions and (ii) genotypic variation in growth rate and in root surface area results in different radiocaesium levels in soil grown plants.

According to the first hypothesis, genotypic differences in K levels will correlate with differences in radiocaesium levels. Since cationic composition of the nutritive solution affects the Cs/K observed ratio of a plant (Cline and Hungate, 1960), root environmental conditions must be controlled appropriately, a condition easily achieved in hydroponics.

The second hypothesis is based on the fact that the caesium mobility in soil is low. Hence, the caesium flux to the roots can be below their caesium interception potential. In this scenario, radiocaesium levels in the plant are strongly influenced by the growth rate of the plant (through the growth dilution effect) and the root surface area.

Both hypotheses were tested and the results are reported. The first hypothesis was tested with two crops of contrasting potassium levels (spinach and wheat). Different ionic compositions of the nutrient solutions were applied in order to assess the generality of this hypothesis. The second hypothesis was tested by growing these crops in a potted soil contaminated with radiocaesium. Using phytotron facilities, this experiment was carried out at two contrasting climatic conditions in order to include growth rate effects on radiocaesium levels for each species.

Materials and methods

Nutrient solution experiment

Eight day old seedlings of spinach (*Spinacia oleracea* L., cv. Subito) and wheat (*Triticum aestivum*, cv. Tonic) were transferred from perlite to culture tanks containing continuously aerated solutions (1L/plant). Plants were grown in walk-in growth chambers (Weiss 18'SP/+5JU-PA) with a 12 hrs day/night cycle.

Day/night temperatures were 18.5°C/14.5°C and the relative humidity was kept constant at 75 %. Photosynthetic photon flux density at canopy height was 660 $\mu mol\ m^{-2}s^{-1}$.

Eight nutritional scenarios were applied in which K, Ca, Mg and NH_4 concentrations were varied keeping total salt concentration and relative anion concentrations invariant (Table 1). ^{137}Cs was added at a level of 10 Bq/ml. During the experiment cationic concentrations were controlled to remain within 20 % of initial values. Initial pH of all solutions was 6.15 ± 0.10.

In total, 40 plants of each species were grown at each nutritional scenario. Twenty six plants were harvested at 15 (wheat) and 16 (spinach) days after sowing, the remaining plants at 19 (wheat) and 20 (spinach) days after sowing.

Nutritional effects on relative growth rate in the exponential growth stage were marginal, the average relative growth rate being $0.40 \pm 0.03\ day^{-1}$ (spinach) and $0.023 \pm 0.01\ day^{-1}$ (wheat). Results are reported in terms of transfer factor (TF) defined as the ratio of ^{137}Cs in oven-dried (70°C) plant tissue to solution levels. At harvest, plants were divided into shoot and root, rinsing roots with deionized water. Plant tissue was digested in HNO_3-$HClO_4$-H_2SO_4 mixtures and ^{137}Cs activity in the digest was measured in a Minaxi 5530 auto-gamma counter.

Potted soil experiment

A top layer (0-30 cm) of a farmer's field was collected from Kalmthout (Belgium). The soil developed from a podzol by continued additions of organic matter. Chemical characteristics of this soil are reported elsewhere (De Rycke, 1992). The soil was dried to a moisture content of 1.5 % and sieved (2 mm). The soil was moistened with a nutrient solution (n°8 in Table 1) to a final moisture content of 18.0 % (about 80 % of field capacity). The soil was contaminated with a ^{137}Cs spike (259 Bq/g dry soil) and thoroughly mixed. One L pots were filled with 1.14 kg soil, covered and incubated for 21 days at 25°C. Seeds were germinated for 1 day (wheat) and 2 days (spinach) on moistened paper and transferred to the pots (1 per pot). The soil surface was covered with 100 g of polyethylene beads. Plants were grown at two climatic conditions : one set at the conditions described

above ('summer') and the other one ('winter') at equal temperature and humidity conditions but only 1/3 of the light intensity. Day length in the 'winter' conditions was reduced to 8 hrs. In both conditions, 16 plants of each species were grown. Soil moisture content was adjusted daily with alternating deionized water or a 1/2 diluted nutrient solution (n° 8, Table 1) additions. For each combination of species and climate, plants were harvested twice. Ten plants were removed at the first harvest and 6 at the second. After removing the shoot, roots were handpicked from soil and washed. Two root-free soil samples were taken per pot. The soil solution was isolated with a centrifugation technique (Mubarak and Olsen, 1976) and the ^{137}Cs activity was measured. ^{137}Cs activity in dried root and shoot samples was assessed as described above.

Results and discussion

Nutrient solution experiment

The results listed in Table 2 falsified the first hypothesis cited above. At any nutrition scenario, the Cs/K ratio varied extensively between spinach and wheat : the K content of spinach is on average 1.80 times more than that of wheat whereas the ^{137}Cs level of spinach is on average only 1.05 times more than that of wheat. However, the ^{137}Cs level in the shoot tissue is always higher (on average 1.39 times) in spinach than in wheat although the potassium distribution over shoot and root tissue is more similar in both crops (details not shown). The ionic composition of the nutrient medium strongly affects the transfer factor (Table 2). A mechanistic and quantitative relationship for this interionic effect has been given recently (Smolders et al. 1993, unpublished). According to that relationship, uptake of the radionuclide depends on its loading in the root exchange complex at the plasmalemma. As monocots are reported to have a lower root cation exchange capacity than dicots, corresponding to a higher preference for monovalent ions (Wacquant, 1977), the fractional loading of ^{137}Cs can be higher in the cortical free space of wheat than that of spinach. On the other hand, the efficiency by which monovalent ions are transported from the cortical free space into the root cells, is lower in wheat than in spinach as suggested by the different potassium levels in both plants. Both opposing trends could result in almost equal transfer factors in both plants. This interpretation however should be strengthened by additional experiments as the measurement of the ^{137}Cs loading in the root exchange complex of both plants.

Potted soil experiment

The ^{137}Cs content of wheat is mostly higher than that of spinach at equal climatic conditions and harvests (Table 3). The radionuclide content decreased during development and this tendency was more pronounced in 'summer' than in 'winter'.

The 'in situ' solid/liquid distribution coefficient K_D of Cs was estimated to be about 50 ml/g and soil contamination was carried out in order to obtain 5.5 Bq in 1 ml soil solution. This level was found at the first harvest (13 days after sowing, Table 3). At later harvests, a steep decrease of those levels were found and large variances between replicates were observed up to 17 days after transplanting.

The steep decrease of ^{137}Cs levels in soil solution is unexpected : (i) the amount of activity removed by plant uptake is very small (the highest uptake is still below 10 % of initial ^{137}Cs amounts present in soil solution and below 0.05 % of initial ^{137}Cs amounts present in the pot) and (ii) estimated depletion of potassium levels in soil solution cannot explain a more-than-doubling of the K_D (calculation details not shown). No explanation can be given for the (most probable) sudden increase of the K_D around 13-17 days after transplanting which followed 21 days of incubation at 25°C.

Unfortunately, as the chronological changes of the ^{137}Cs levels in soil solution were not quantified in detail, no conclusions can be made on the genotypic and climatic effects on the ^{137}Cs transfer factor.

A final remark is that the transfer factor, as defined in Table 3 can only be compared with the transfer factors of Table 2 if radionuclide levels in soil solution are constant during development. If it is assumed that these are stable up to 13 days after transplanting ($\pm$ 5.5 Bq/ml as expected), the first transfer factor we observed at 13 days after transplanting is of the same order of magnitude of that of a hydroponically grown plant (Table 2). This suggests that caesium transport in soil does not strongly limits plant uptake.

In conclusion, the differences in K levels between spinach and wheat do not correlate with equal differences in radiocaesium levels when both crops are grown at identical root ionic environments. No conclusion can be furthermore made on genotypic or climatic effects on caesium uptake from soil since radiocaesium levels in the soil solution of the experiment reported here varied extensively in an unknown way.

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Smolders E and Merckx R 1993 Proc. C.E.C. "REACT" Workshop (Brussels, Oct. 1991) Sci. Total Environ., in press.

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Table 1 . Cationic concentration in (mM) for the eight nutritional scenarios studied. Anionic concentrations (in mM) : 5.92 (NO_3^-), 0.5 ($H_2PO_4^-$), 1.73 (SO_4^{2-}), 0.5 (Cl^-), Fe EDTA and trace elements were added as described elsewhere (Smolders et al. 1991).

Scenario	$[K^+]$	$[Ca^{2+}]$	$[Mg^{2+}]$	$[NH_4^+]$
1	10.06	0.19	0 .08	0.00
2	5.56	0.28	0.12	4.24
3	1.06	0.38	0.15	8.47
4	0.79	1 98	0.80	4.24
5	0.53	3.58	1.45	0.00
6	5.30	1.88	0.77	0.00
7	3.18	1.13	0.46	4.24
8	3.59	2.49	1 01	0.00

Table 2 . ^{137}Cs Transfer Factors (TF) and potassium content of hydroponically grown spinach and wheat plants. Values refer to the total plant (shoot + root) and are averages of two independent measurements. Standard deviations are given in parenthesis. Plant age at harvest is 19 days (wheat) and 20 (spinach) at which dry weight varies between 0.2 and 0.3 g.

Scenario	spinach		wheat	
	TF(ml/g)	K (meq/g)	T Fml/g)	K (meq/g)
1	117.0(5.7)	2.9(0 2)	95.8(3.7)	1.5(0.1)
2	90.6(1.5)	2.2(0.3)	65.6(2.1)	1.4(0.1)
3	87.6(1.6)	1.9(0.2)	84.4(0.5)	1.2(0.1)
4	58.4(0.9)	2 2(0.1)	54.4(3.9)	1.2(0.1)
5	49.2(4.5)	2.7(0.1)	79.6(8 9)	1.3(0.1)
6	58.1(0.9)	2.6(0.3)	63.9(1 1)	1.5(0.1)
7	60.9(2.4)	2.2(0.2)	51.9(3.0)	1.3(0 1)
8	54.2(2.5)	2.8(0 1)	62.4(5.9)	1.4(0.1)

Table 3 . ^{137}Cs activity in spinach and wheat plants and in the soil solution collected at harvest. Standard deviations are given in parenthesis. ^{137} activity in plants refers to the total plant (shoot + root). 'Transfer factor' is defined here as the ratio of ^{137}Cs in dried plant tissue to soil solution.

Crop	Age (day after transpl.)	Plant dry weight(g)	^{137}Cs,plant (Bq/g)	^{137}Cs,soil (Bq/ml)	"TF" (ml/g)
spinach					
'summer'	17	0.193(.430)	196(44)	2 7(1.1)	73
	23	0.444(160)	115(15)	1.4(0 2)	82
'winter'	19	0.050(.010)	184(15)	2.4(0 5)	77
	24	0 064(.015)	181(3)	2 2(0 1)	82
wheat					
'summer'	13	0.246(045)	296(38)	5.9(0.7)	50
	17	0.672(112)	140(12)	2.2(0.9)	64
'winter'	19	0.143(.028)	226(56)	1 8(0.2)	126
	24	0.195(.030)	172(12)	1 8(0.4)	96

Head of project 5: Dr. Staunton

II. Objectives for the reporting period

1. Development of mechanistic model to describe the bioavailability of radiocaesium in soils.

- identification of the limitations of the prototype model (Kirk & Staunton, J. Soil Sci. 1989)

- selection of geometry, boundary conditions, etc.

2. Investigation of the mechanism of the aging process of adsorbed caesium in order to optimise its mathemetical description in the model

3. Study of the effect of various experimental conditions on measured values of Kd.

Comparison of soil-extracted and reference clays

III. Progress achieved including publications

1. Model Development

The model is being kept as simple as possible, but designed so that other factors can be tested at later stages. The following factors are taken into account :

Uptake by plant roots from soil solution at the root-solution interface
Movement to the root-solution interface by diffusion in the solution phase
Rapid, reversible adsorption onto the surfaces of soil constituents. Linear adsorption isotherm, described by Kd.
Slow, reversible reaction with the surfaces of soil constituents (aging or fixation), described by first order kinetics.

Roots are assumed to be cylinders which exploit cylindrical volumes of soil. Total uptake during a growing season is summed over groups of roots with differing uptake properties and/or rooting densities in soil layers with differing concentrations of caesium. Root uptake is not be assumed to be limited by steady state diffusive flux to the root-solution interface, as was the case in the prototype model, since this cannot be justified for a growing root system. Vertical distribution is calculated in a subroutine at the beginning of each simulated growing season and assumed not to vary during the growing season. The soil is divided into bands with fairly uniform caesium concentrations and rooting densites and uptake calculated for each.

2. Development of a new technique to follow the aging process

One postulated mechanism for slow fixation of adsorbed caesium is the movement of the cation to selective sites, possibly interlayer sites, followed by partial collapse of the interlayer spacing. The extent of such a reaction would thus depend on both time and temperature. The effect of incubation temperature (5, 20 or 35°C) and period (hour, day, week or month) in two reference clays, Wyoming montmorillonite and Le Puy illite, have been studied.

An experimental procedure has been developed which avoids the experimental artefacts often associated with desorption experiments (arising from repeated phase separation and resuspension, and differences between the composition of the adsorption and desorption solutions). After incubation of the clay with a known amount of stable caesium in dilute suspension, a trace amount of radiocaesium is added. The distribution of isotope after a short period (2 h) is a measure of the proportion of adsorbed stable caesium which is readily exchangeable. When the concentraion of stable caesium is very low there is also further adsorption.

No effect of incubation has been found with montmorillonite at any temperature and at any level of caesium in the range 10^{-8}-10^{-5}M. However preliminary results for illite indictate that the apparent Kd increases as the incubation period increases when the clay is saturated in calcium and to a lesser extent when saturated in sodium. No significant effect is observed with the clay is saturated in potassium.

Apparent Kd values (dm^3kg^{-1}) of ^{137}Cs on Ca-illite after incubation in solutions of stable Cs at 5°C for varying periods.

	Incubation period (h)			
[Cs] (M)	2	20	140	720
10^{-8}	**181800**	**191100**	**292500**	**692600**
10^{-7}	**103300**	**117700**	**168600**	**304300**
10^{-6}	**6310**	**6650**	**8360**	**10380**

3. Effect of experimental conditions on Kd

The Kd values of radiocaesium on contrasting soils have been measured in suspension. The concentration of stable caesium and of ^{137}Cs, the nature of the background solution (deionised water or 0.01M $CaCl_2$) and the solid:solution ratio have been varied. For each of the soils Kd decreases with increasing caesium concentration **(Figure 1)**, even when no carrier is added (10-100 $kBq.ml^{-1}$, 1 $g.ml^{-1}$). The adsorption isotherms are thus not linear, and the true distribution coefficient in a contaminated soil could be much higher than that measured in the laboratory. Kd values are consistantly lower when measured in a background electrolyte of 0.01M $CaCl_2$. This agrees well with data obtained from reference clays where the Kd was found to decrease with increasing ionic strength **(Figure 2)**. The effect of soil:solution ratio is complex. In general increasing soil:solution ratio for a given initial solution concentration in radiocaesium leads to increasing Kd. This is the result of changing equilibrium solution composition and more importantly, since the adsorption isotherm is nonlinear, increasing the amount of soil to which a given activity of ^{137}Cs is added is equivalent to decreasing the initial concentration of caesium at constant soil:solution ratio.

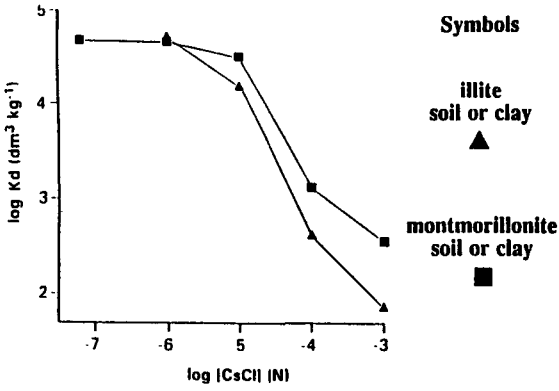

Figure 1. Effect of concentration in CsCl on the Kd in soils

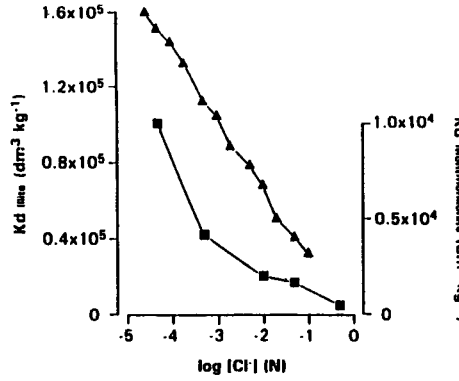

Figure 2. Effect of salt concentration on the Kd in clays

A comparison of the standard values of Kd measured for two soils containing predominantly montmorillonite or illitic material and the corresponding reference clays indicates that soil clays behave differently to pure clay minerals. Neither the presence of organic matter nor the composition of the exchange complex (the dominant cations in both soils are calcium and magnesium) can explain the difference. One possibility may be that the exchange properties of natural soils are dominated by trace amounts of illitic material which is not detected by routine mineralogical analysis.

Kd (dm^3kg^{-1})		Montmorillonite	Illite
Clay	Ca	**2010**	**244000**
	Na	**3220**	**34200**
	K	**730**	**4300**
Soil		**49400**	**52000**

Publications

STAUNTON S. & ROUBAUD M. 1993. Measurement of the adsorption of radiocaesium on clays: factors affecting the extrapolation to *in situ* conditions. Mém. Soc. Géol. France, 162, 7p.

STAUNTON S. & ROUBAUD M. Adsorption of radiocaesium on montmorillonite and illite: effect of charge compensating cation, ionic strength, concentration of potassium, caesium and fulvic acid. In preparation.

STAUNTON S. On the prediction of the distribution coefficient of ^{137}Csin soils from clay mineralogy. Abstract submitted to 15th World Congress of Soil Science, Acapulco Mexico, 10-16 July 1994.

Head of project 6: Dr. Berthelin

II. Objectives for the reporting period

Several objectives were carried on : first a more complete bibliography study has confirmed the interest of the possible role of soil microorganisms in the mobility of cæsium through their involvement in the transformation of organic and mineral soil constituents and their properties (e. g. accumulation, exchange, proton production...). That includes all the interactions between organic matter and this radionuclide, and the different reactions to be taken into account so as to study these interactions in the soil system.

The second objective concerns the developpement of experimental methods and design that are used in the experiments. A semi continuous flow perfusion experimental model was adapted to the present study. It was also decide to use titrimetric determinations of Cs-organic matter complexes. The results includ also the choice sampling and the characterisation of the soils selected. A rendzina (typic rendoll) and an orthic luvisol (typic hapludalf) were selected, because of their important representation around the french nuclear sites. At least a common soil or a similar soil than those used by the other contractants will be utilized.

III. Progress achieved including publications

The bibliography that has been made, confirm the interest of studying the role of the microorganisms and the organic matter in the mobility and availability of radionuclides. It allows to underline some of the natural conditions in which organic matter and biological mechanisms can play an important role in the cæsium behaviour in the soils. This next period will be used to follow the experiment and to obtain an interpretation of the results of the different experiments mentionned in the previous paragraph and presented more completely in the following more detailed report. These results would indicate in what manner the microorganisms can be involved in the complexation, the release and the storage of cæsium through a soil profile. The different results obtained will allow to consider other ways of research that will be approached later such as : the specific retention of radionuclides in some soil horizons, the bounds between Cs and organo-mineral complexes, and in fact the establishment of closer relations between the different types of soils in order to foresee the behaviour of Cs in natural conditions.

The principal results and the progress obtained during the last reporting period, concern essentially as mentionned previously in the summary : a more complete bibliography, the developpement and perfection of experimental methodes and design that are used in the experiments including the choice sampling and characterisation ot the selected soils.

I. Bibliographic study : why microbial activity seems important to be studied on the migration and behaviour of Cs in soils :

Most of the works of the last decade about the interactions of the radionuclides and more particularly cæsium with the constituents of the soils, are dealing mainly with the mineral constituents (Cornell, 1992 ; Wagner and Czurda, 1990), and with its distribution in the soils (Bunzl and al, 1988 ; Davies and Shaw, 1993). The interactions between radionuclides and organic matter are rather little studied (Valcke and Cremers, 1992 ; Bovard et al, 1968 ; Bittel and Lehr, 1968 ; Witkamp, 1968).

However in pedological processes, biological mechanisms, that include the role of organic matter and the role of all the soil living community are playing an important role in the biogeochemical cycles : chelation, complexation, migration through a profile, storage in the upper horizons, bioaccumulation in microorganisms, increasing availability for the plant roots... This last point is very important as it takes place at the beginning of the contamination of the food chain in the case of radionuclides. The behaviours of Cs and K can be compared because of their similar characteristics and properties (ionic ray, weight, same chemical group). This allow to make some assumptions about a comparable behaviour in the soil. This can be considered more easily if the biogeochemical cycle of K is well known. For example even if it does not concern a great part of it, cæsium retained in the clay can be exchanged with K, and so be released in the environment.

When cæsium is deposited on the surface of the soils, at least 80% bounds with the mineral constituents, as it has a great affinity for clays and illite in particular. These bounds concern essentially the FES, Frayed Edges Sites (Cremers, 1988 and 1993) and a little proportion seems to be irreversibly retrogradated. It is why Cs remains in the first centimeters of the soil and seems to have low mobility. For instance 20 and 25% respectively in an alluvial soil and a podzolic soil are concerned by the leaching and for this fraction a great part is retained between 5 and 10 cm (Bovard et al, 1968).

But soils of high organic matter and low clay content pose some problems because organic matter can adsorb a great part of the Cs deposited. And even if the proportion of cæsium linked to the organic matter is not quantitatively very important in the most case of soils, we have to not forget

that this fraction represents the more available fraction for the plants, directly or after a first release in particular by the microorganisms and their biological activity. For these reasons it is interesting to present and discuss what we are today knowing about the interactions Cs-organic matter.

For Bovard et al. (1968) Cs is able to link itself with the organic matter in such a repartition :

- 75 to 85% is exchangeable
- 15 to 25% is not directly exchangeable, but can become exchangeable
- 0,5% can migrate as fulvate
- 0,5 to 1% is stored in the organic matter

For the greatest part Cs linked with the organic matter remains exchangeable but fulvic acids are able to stock some fission products in the B_h horizon of podzolic soils.

The availability of radionuclides for plants is associated with the degree of their passage in the soil solution, and depends also on the different molecular weight of their water soluble organic compounds (Agapkina, 1991).

In some low clay content soils, flush of ammonia through the system of FES (Cremers and al, 1988) could displace some of the sorbed Cs into soil water, and hence into streams (Hilton et al., 1993). At this stage we can suppose that organic matter will be able to form water soluble complexes with cæsium.

Birch and Bachofen (1990) are giving examples of the effects of microorganisms on the environmental mobility of radionuclides : complexation with organic compounds of different weight and origin (microbial exudates and constituents), biosorption, bioaccumulation, precipitation. These review gives some interesting bases for the specific studies of the cæsium and for future researches on the mobility induced in the soils by those phenomenons. Radiocæsium can form complexes with artificial organic components such as valinomycin, nigericin and other similar molecules (Desmet et al., 1991). Cyanoferrate can also form a very strong complexe with Cs (Vreman et al., 1992). It will be perhaps interesting to study this kind of complexation of the cæsium with such compounds as a way to desorb Cs from the soils. It will also be a way of chemical remediation with synthetic complexes or of biodecontamination by using microorganisms able to produce such compounds.

Literature gives some examples of the role played by microorganims in the cycle of radionuclides and particularly in their release in the ecosystem. We will just here give some references : West et al. (1987) showed the effect of sulfate reducing bacteria on the ^{137}Cs sorption onto calcium monmorillonite, a potential backfill material. These microorganisms weather the rock material and make the nuclide more mobile, in some cases by up to 2 or 3 orders of magnitude. Differences about Cs mobility between laboratory studies and field assays were noted (Walton and Merrit, 1980) and were there attributed for a great part to the contribution of microorganisms (Champ and Merritt, 1981).

In a first conclusion of this bibliographic study, mineral fraction appears us preponderant in the behaviour of Cs in the soils but organic matter and microbial activity are able to play an important

role in its evolution : temporary linkage, migration, storage and availability. Organic matter will certainly not play a role of specific retention, but the retention that can occur is to be taken into account in quantity and quality. The possible role of microorganisms remains to be determined. In order to study the behaviour of Cs in the soils it will be also interesting to prepare an organo-mineral complex and to study in function of the Cs concentration used the interactions involved.

A second important conclusion is suggested by some other observations. In a study about the distribution of radiocæsium in the organic and mineral fractions of pasture soils and their subsequent transfer to grasses, D'Souza et al. (1968) remarked that in the case of soils of high organic matter and low clay content, the uptake of Cs by grasses was at a slower rate up to the third cutting, then the bioavailability again increases. This phenomenon may be due to many factors including moisture supply. But this indicate also that there is no stability with time and that as the soil functionning the dynamic of the release of the radiocæsium is always in evolution and can be under the influence of biogeocycling processes.

Cs present in the mineral fraction can get soluble and mobile again under chemical, physical or biological weathering processes influenced by different characteristics of the soil such as pH, aeration, organic matter/mineral fraction ratio, etc... It is possible that even if a small amount of illite is present in the soil, organic molecules could prevent the developpment of irreversible sorption (Livens et al., 1991).

All these last observations and considerations suggest that it is important to study the processes of remobilisation of Cs involving microbial activity (weathering, dissolution, retention, deposit...) from the mineral soil constituents.

II. Developpement of experimental methods and design :

In order to study the role of the microorganisms on the mobility, lixiviation, retention of cæsium through the soil, we are using semi-continuous flow perfusion devices. The experimental design is described here very briefly, all the details will be presented in the final report. Because this experiment is still in his first step we will not present here result concerning the Cs leaching.

For these first experiments two soils were selected : a rendzina or typic rendoll and an orthic luvisol or typic hapludalf. The principal characteristics of these soils are presented in table I.

Soils	pH	Organic matter (%)	CEC (meq/100g)
Rendzina	7,4	15	45,5
Orthic luvisol	6,4	5,2	14,5

Table I : Principal characteristics of the horizon A (0-10 cm) of both soils selected.

The soils are sieved at 2 mm and saturated with a solution of cæsium chloride. Different analyses are made in order to quantify the amounts of cæsium adsorbed on the soils. Cesium is measured with an atomic absorption spectrometer and a mass spectrometer. Columns are filled with the cæsium saturated soil and are subjected to percolation.

Four types of treatments are performed : sterile conditions (without microorganisms) or biotic conditions (with microorganims). For each treatment (biotic or abiotic) two solutions of percolation are used : rainy water or nutritive solution. Table II presents the different treatments performed for each soil.

Parameters	Abiotic conditions	Biotic conditions
Rainy water	4	4
Nutritive solution	4	4

Table II : Number of replicates for each treatment and each soil.

The rainy water is obtained by bubling CO_2 in water. The nutritive solution is a solution of glucose in order to stimulate the microorganisms. Abiotic conditions are obtained by adding an antimicrobial compounds in the percolating solution . Biotic conditions are obtained by inoculation of the column with a suspension of microorganims of the corresponding soil.

Different analyses are performed :

- in the percolated solution : measurement of pH, concentration of Cs and others elements, research of organic compounds and microbial metabolites
- microbiological determination in the different treatments
- observations of the constituents of the soil in order to localise the cæsium and to control its behaviour by soils analysis

Solution of cæsium chloride is used in order to simulate the input of ionic form of Cs on the soil. With the experimental design previously described, we do not take into account the complexity of the soil solution because we are starting with a saturated soil. This homoionic condition is not representative of the in situ conditions, but at present we first intend to study in a short time the influence of the micoorganisms on the leaching of cæsium and weathering of constituents bearinfg cæsium. Later we will done similar experiments but with non homoionic non saturated soil by using radioactive tracer and isotopic technics. A rather good adequation between the results obtained with column experiments and fields studies can be awaited because Schimmack and Bunzl (1992) have shown that the presence of a layer of organic material on a mineral soil in the column experiments influence the migration of radiocæsium in a parabrown earth spruce.

Others experiments and analysis are too performed and concern the acid titration of complexes established between cæsium and organic acids in order to know the possibilities of formation of

these complexes and the conditions of their stabilities. These experiments will give some complementary results.

Our laboratory has an experience in the study of the interaction between microorganims and minerals in soils, for instance concerning the microbial rhizosphere weathering and the behaviour of K in different forest soils(Berthelin et al. 1991 ; Leyval anf Berthelin, 1991 ; Berthelin and Leyval, 1982). But it has also an experience in the study of the radiocnuclides elements and especially results have been performed on the biogeochemical cycle of uranium in different soils (Berthelin et al., 1988.; Guéniot et al. 1988 ; Munier-Lamy et al., 1991 and 1986). Microrganisms have been proved to play a role on the dissolution and the deposit of uranium in soils. This work will be presented at the next GSF workshop in Udine in next october. The summary of this communication is enclosed. Another incidence of these results is the fact that Cs, as observed in the case of the accident of Tchernobyl, can be bound to uranium oxides (Cremers, 1993), that is not directly available for the plants but can be weather in such form.

The final objective of this work on the cæsium is to study its biogeochemical cycle and to determine the involvement of microorganisms in bioaccumulation, weathering and biosorption processes.

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Head of project 7: N.Mocanu

II. Objectives of the reporting period

The present reporting period includes the preparative operations of the experiment on the evaluation of agricultural soil-based countermeasures to reduce soil-to-plant transfer, using a modified batch equilibrium technique. According to the preparative stage of the experiment, the main objectives took aim at:

(a) getting preliminary informations about soil types in Romanian agricultural areas and sampling some of them considered as representative;

(b) preparation of pots and space for the storage of radioactive contaminated soils used in experiment;

(c) physico-chemical characterization of the collected soils;

(d) artificial contamination of collected soils with ^{137}Cs and ^{90}Sr.

III. Progress achieved including publications

1. Brief description of soils selected for experiment.

After a preliminary investigation on the large variety of soils existing in Romania, especially in Southern part which is the most important agricultural area of the country for cereal and vegetable cultures, three soil types were selected taking into account their extent and importance for agricultural use:

(a) an alluvial soil (sandy-loamy) which usually characterizes the floodable meadows placed in immediate vicinity of the rivers; the areas with this soil type are largely used in agriculture having a high fertility and due to their easy access to the irrigation with water from rivers during the rainless summers.

(b) a brown reddish soil (clayey-loamy) which is an argillaceous soil formed on sandy and loess deposits; usually is recommended for cereal cultures.

(c) a "green house" soil based on old manure, having a high percentage of organic matter, considered as organic soil.

2. Physico-chemical characteristics of soils.

The physical and chemical analyses of the selected soils have

included granulometry, organic and mineral matter contents, pH, cation exchange capacity, exchangeable basic cations (K^+, Na^+, Ca^{2+}, Mg^{2+}), H^+, basic cation saturation. The results are shown in Table 1.

Table 1: Physical and chemical properties of soils collected for experiment

Soil type	Sandy-loamy	Clayey-loamy	Organic
Organic matter (%)	4.8	3.6	52.4
Mineral matter (%)	95.2	96.4	47.6
Coarse sand (%)[1] [2.0-0.2 mm]	6.4	3.2	
Fine sand (%)[1] [0.2-0.02 mm]	54.4	32.4	
Silt (%)[1] [0.02-0.002 mm]	20.5	30.8	
Clay (%)[1] [< 0.002 mm]	18.7	33.6	
pH (H_2O)	7.8	7.5	8.5
pH (KCl)	7.2	6.7	7.8
CEC (meq/100g)[2,3]	16.29	22.25	68.20
Ex.K^+ (meq/100g)[2]	0.59	0.51	19.17
Ex.Na^+ (meq/100g)[2]	0.54	0.24	6.05
Ex.Ca^{2+} (meq/100g)[2]	13.57	18.00	42.98*
Ex.Mg^{2+} (meq/100g)[2]	1.59	3.12	42.98*
Ex.H^+ (meq/100g)[2]	-	0.38	-
BCS (%)[4]	100.0	98.3	100.0

[1] percent of mineral fraction only; [2] dry weight;
[3] cation exchange capacity; [4] basic cation saturation;
* Ex.Ca^{2+} + Ex.Mg^{2+}

The methods used to determine physical and chemical characteristics of soils are standardized methods regularly in use in specialized agricultural chemistry laboratories.

Cation exchange capacity of alluvial and "green house" (organic) soils were determined using the ammonium saturation method recommended for soils rich in carbonates (including a previous decomposition of $CaCO_3$ with acetic acid 1n and a subsequent soil saturation with NH_4^+ by the use of an ammonium

acetate solution 1n, pH 7.0). In these soils high concentrations of total calcium were found: 100 meq/100g in alluvial soil and 135 meq/100g in "green house" soil. Cation exchange capacity of brown reddish soil was determined as the sum of exchangeable basic cations and H^+.

3. Preparation of pots and place for the storage of radioactive contaminated soils.

The pots for radioactive contaminated soils are plastic barrels (41 centimetres in diameter) doubled with polyethylene bag, having on the bottom a drainage pipe connected to a plastic flask wich to collect a possible release of water in excess. The place chosen for storage of pots with radioactive contaminated soils is a closed space with controlled access, used as store for radioactive sources. There are three pots with radioactive contaminated soils and other three pots filled with non-radioactive soils used as controls.

4. Radioactive contamination of soils for experiment.

The soils for the experiment have been artificially contaminated with ^{137}Cs in the form of chloride and ^{90}Sr in the form of nitrate. Diluted radioactive solutions were prepared with rain water to avoid possible strong modifications of ionic equilibria which could appear by using the distilled water. The contamination levels of soils, 100 kBq $^{137}CsCl$/kg dry soil and 10 kBq $^{90}Sr(NO_3)_2$/ kg dry soil, were established so that, at equilibrium between applied radionuclides and soils, to get in soil solutions activities of tens to hundreds Bq per litre for ^{137}Cs and activities of hundreds Bq per litre for ^{90}Sr (taking into account the K_d values found in literature for ^{137}Cs and ^{90}Sr, respectively).

To get as much as possible an uniform distribution of radionuclides in soils, small portions (1kg dry soil) were contaminated using equal quantities of radioactive solutions with the same radionuclide concentration (specific activity). After that, the soil portions were put together and mixed in pot, for each type of soil.

The soil humidity will be permanently controlled, maintaining the soils at field capacity by hand watering with rain water collected previously.

To check up reaching the equilibrium between applied radionuclides and soils, the concentrations of radionuclides

and excengeable cations (i.e. K^+ and Ca^{2+}) will be measured in soil solutions. After reaching the equilibrium between applied radionuclides and soils, the effects of different chemical treatments on radionuclide availability from contaminated soils will be evaluated by using a modified batch equilibrium technique, as it was presented in a recent paper [Nisbet A.F., Mocanu N., Shaw S.: Modified batch equilibrium technique - a rapid laboratory method to predict the effects of agrochemical countermeasures on solid-liquid equilibria in soils contaminated with radiocaesium and radiostrontium. Science of the Total Environment (in press)].

Progress Report

Contract: FI3P-CT920022 Sector: A23

Title: Investigations and modelling of the dynamics of environmental HT/HTO/OBT levels resulting from tritium releases.

1)	Bunnenberg	NIR
2)	Belot	CEA - Cadarache
3)	Kim	Univ. München - Technische
4)	Dertinger	KFK

I. Summary of Project Global Objectives and Achievements

The operation of fuel reprocessing and tritium extraction plants, but even more so, the progress in the technical realization of nuclear fusion involving the handling and storage of several kilograms of tritium within a single facility stress the necessity to predict time courses and maximum levels of tritium concentrations in environmental compartments after short-term and routine emissions. In order to reduce the uncertainty factor of existing tritium dose models, two essential topics on environmental tritium behaviour have been selected for intensive studies in the framework of this joint project:

a. Parameterization of tritium reemission after HT or HTO deposition to soils representing a secondary source of air-HTO and
b. Plant-specific variations and dynamics of tritium uptake, OBT formation and translocation in biological systems.

Both topics have been split among two institutes each, to ensure complete coverage of all associated aspects. This concept also provides the links between laboratory and open field results as well as between transient and steady state observations for both topics.

Single-parameter studies on the time course of HTO reemission rates have been performed with help of a wind-tunnel/soil-column arrangement under controlled conditions (NIR-Hannover). The long-lasting controversy, whether or not HTO reemission is coupled to H_2O reemission (evaporation) has been solved to the extent that the potential reemission of both, HTO and H_2O, is equally depending on soil physical and micro-meteorological parameters, whereas the effective reemissions of the two types of molecules is solely governed by their relative abundances in soil moisture and air humidity. Consequently, the shape of the concentration profile of air-HTO is part of the small scale reemission experiments in the field, where deviations from the commonly used Gaussian profile have been determined in the collection of the reemission plume (CEA-Fontenay aux Roses). Two recent joint experiments from grass-covered and bare soils have yielded high time-resolution reemission rates from plume sampling (CEA) and mean reemission rates from soil-profile determinations (NIR). This bi-directional approach also renders partial parameter separation under field conditions.

Common analytical errors in the analysis of tritium compounds in biological systems due to isotopic effects and exchange processes during separation procedures have been identified in detail and quantified for a standard procedure for correction purposes (TUM-München). On this basis equilibrium-state OBT-TWT fractionation factors (R-values) have been determined for a C_3 and a C_4-plant both being well below unity. Their differences can be explained by the specific metabolic differences of those species. Refinement of the diurnal course of HTO leaf uptake was achieved by gas exchange measurements, representing the basis for the dynamics of OBT formation in plants (KfK-Karlsruhe). The studies also include the previously less considered pathway of OBT translocation into edible plant organs. HTO uptake, OBT formation and OBT translocation have been combined in a detailed compartment model for introduction into a tritium dose computer code.

Head of project 1: Dr. Bunnenberg

II. Objectives for the reporting period

In order to improve our understanding of tritium reemission from soils after HT or HTO deposition and to refine existing tritium dose models accordingly, two types of investigations are performed: single-parameter studies on the time course of HTO reemission rates with help of a wind-tunnel/soil-column arrangement under controlled conditions and evaluations of HTO profiles after reemission phases in small scale experiments jointly carried out with the CEA research group.
The investigations focused on time courses of HTO reemission rates and total HTO and H_2O losses from soils in relation to the relative abundance of HTO and H_2O in air humidity and soil moisture. Emphasis was put on correspondences between laboratory and open field findings especially with respect to the shapes of the soil HTO profiles and mean rates of HTO and H_2O reemissions.
Both types of experiments will be continued including the aspects of physical soil properties and initial tritium distributions within the soil, aiming at the characterization of HTO availability for reemission and HTO resupply from deeper soil layers. Finally, soil cores taken from the experimental fields will be employed in the wind-tunnel experiments, to obtain detailed correlations between lab and field tests for the transformation of laboratory to open field scales.

III. Progress achieved including publications

1. Methodology

The laboratory part of the investigations is the simulation of HTO reemission processes with help of the combined device of a wind tunnel and a replaceable soil column. During the reemission from homogeneously wetted and HTO-labeled soil columns, air samples are collected in predetermined intervalls between some minutes and several hours by drawing the air through washing flasks, which contain small, tritium-free volumes of water. After each run the soil columns are disassembled in horizontal layers. The air and soil samples yield time resolved HTO reemission rates and vertical soil profiles of moisture and HTO contents.
The HTO determination procedure has been improved with respect to time consumption and significance of the results. With this method water and HTO are extracted from small soil samples, to determine moisture and HTO contents simultaneously. The soil sample is heated to 300°C in an oven under a stream of pure nitrogen, and the exhaust gas is bubbled through a trap (u-tube) filled with water. The uncertainty of activity and moisture determinations are smaller than the size of the symbols of Fig.1, that is below 3%. This error includes variations of small subsamples due to inhomogeneities.

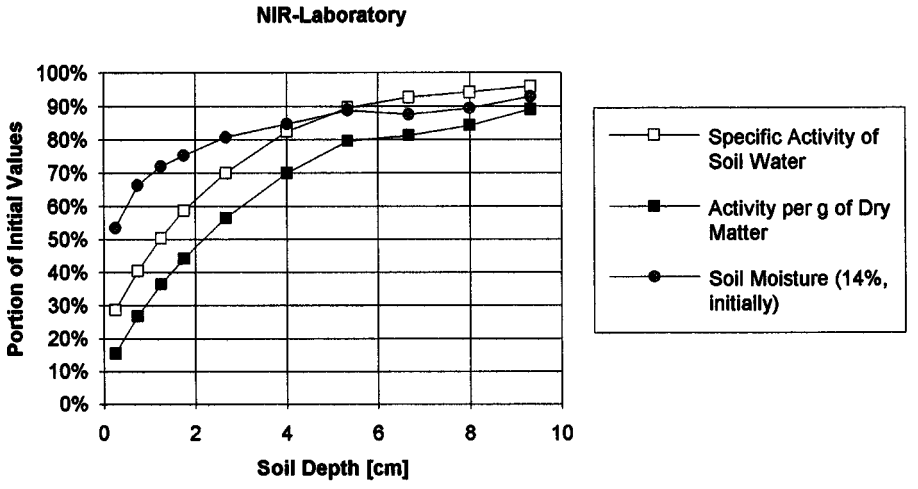
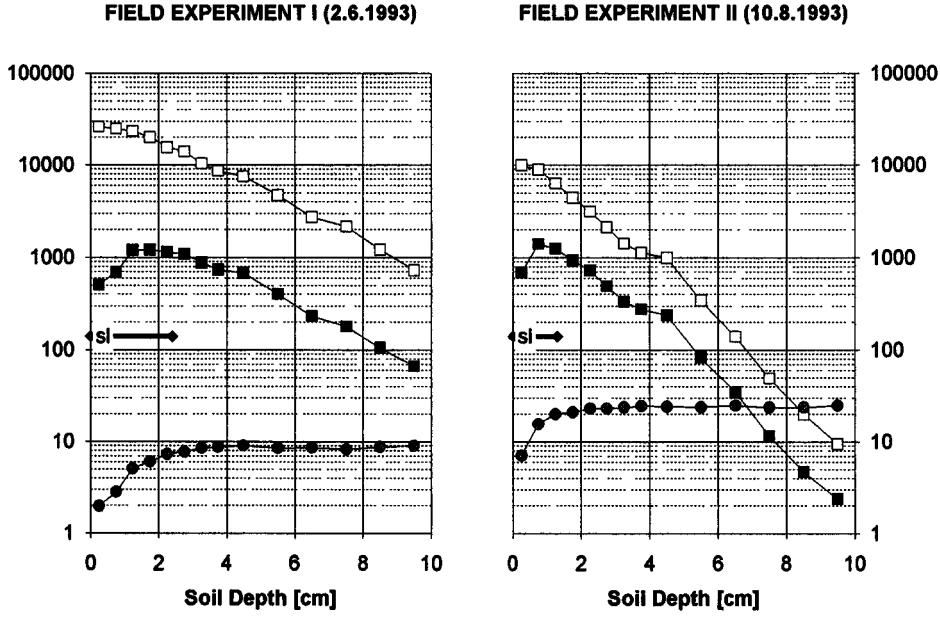


Fig. 1: Depth profiles of activity and moisture after a reemission phase of 5 days into a tritium-free atmosphere with an air humidity of 80%. The soil column was homogeneously labeled with HTO, initially.



Figs. 2, 3: Depth profiles of activity and moisture after HT deposition and reemission of HTO for 3 to 4 hours. (These are joint experiments with the CEA team.)
Open squares: specific activity of soil water [Bq/g],
filled squares: activity concentration [Bq/g(dry matter)],
circles: soil moisture [% of dry matter],
sl: scaling length.

Two field experiments were performed in co-operation with the French team. Soil plots were labeled with tritium via HT deposition and conversion under a removable exposure chamber. At both locations soil cores were taken to analyse the HTO profile of the labeled soil plots after the end of the reemission measurements. In one case the soil was grass covered and agriculturally not manipulated for several years, whereas the soil at the other site was ploughed some days before the experiment.
To combine advantages and to level-out disadvantages, two methods were used to sample the soil profiles. The first is the standard method of taking soil cores with an auger device. This method is very fast, but the spatial resolution is low. The second method is performed with help of a special soil shovel, which allows for taking 5-mm layers with very high precision, but samples may lose water and tritium, as the procedure is time-consuming. However, the measured moisture and activity contents of the samples that were collected by the different methods did not differ by more than 20% and there was no tendency for higher or lower values with either method. The measured differences can be attributed to the natural spatial variations of soil density, moisture content and micro-biological HT to HTO conversion. The soil samples used for the presentation in Figs. 2 and 3 were collected with the soil shovel because of the advantage of obtaining a higher spatial resolution.

2. Results

Typical soil profiles of activity and moisture of initially homogeneously wetted and labeled soil columns in the laboratory are presented in Fig. 1. The air humidity above the soil column was kept at the average level of 80% at room temperature. During the 5-day reemission phase the soil moisture decreased from initially 14%w/w to 7%w/w in the top 5-mm layer and to 12%w/w in the lower half of the soil column. The reduction of the moisture of the lower layers was tested significant, indicating capillary transport of soil water through the whole soil column. The overall loss of moisture from the soil volume was 15%. The evaporation rate was 0.5 mm/day (or 0.13%/h) on the average. At the same time, 30% of the tritium activity of the soil column were lost to the wind tunnel atmosphere, resulting in a pronounced decrease of the specific activity of the soil moisture especially of the upper soil layer. The effect is that the reemission rate of HTO is higher than the evaporation rate of H_2O by a factor of 2, if both rates are expressed as parts of the inital contents of the soil column. This is due to the fact that the air contained H_2O but was tritium-free before reaching the soil column, and it demonstrates the de-coupling of HTO reemission from H_2O evaporation. The HTO reemission rate decreased during the experiment from 0.5%/h (mean of the first 5 hours) to 0.2%/h after 5 days. Discussing the overall effect that the fraction of reemitted HTO is twice the fraction of evaporated water tends to cover the more drastic effect that the specific activity of the evaporating water (ie, the vapour mixture of H_2O and HTO molecules comming from the soil) exceeds the specific activity of the soil water of the top layer by a factor of about 5. This explains the decrease of the specific activity below 30% at the soil surface (Fig. 1).

The activity and moisture profiles of the two soils of the joint field experiments are shown in Figs. 2 and 3. The striking difference to the lab profiles is that the specific activity of the soil moisture is highest in the uppermost soil layer and declines approximately exponentially with depth. The scaling length (sl), i.e. the thickness of the layer representing an activity reduction to 1/e, is 24 mm in the case of Experiment I and 13 mm in the case of Experiment II. The latter is unusually small compared to values found in the literature.

3. Discussion

Although the profiles of the soils of the field experiments were sampled several hours after the end of the HT deposition, the exponential shapes of the specific activity profiles indicate rather constant HT to HTO conversion rates within the soils. Only at the soil surfaces, the slopes are reduced, which is either due to the low soil moisture and thus to lower biochemical conversion or to the activity loss during the preceding reemission process.
In order to compare the HTO reemission from the field and lab soils, the effects of the different shapes of the activity profiles have to be separated from those of the different vapour pressure differences. That is done by assuming that the release of HTO and H_2O molecules from the soil is strictly coupled, which is true if the vapour pressure differences between soil air and atmosphere are the same for both molecules. In this case, the specific activity of the evaporating water equals the specific activity of the soil water of the upper layer. For the lab experiment (Fig. 1) this results in a theoretical HTO reemission rate of 0.13%/h. Assuming the same rate of moisture loss for the field soils (ie, 0.5 mm/day), the HTO reemission would be higher than 0.13%/h by a factor of 4 (Exp. II) or 5 (Exp. I). This increase is due to the high activity content of the first soil layer with respect to the total HTO deposit to the field soils compared to the case of homogeneously distributed HTO within the lab soil.
More theoretical evaluations of the HTO reemission rate resulting from exponentially shaped HTO profiles show that the HTO reemission rate is proportional to the evaporation rate of H_2O and inversely proportional to moisture content, soil density and scaling length of the exponential fit. Ideally, the factor of proportionality equals the ratio of the individual vapour pressure differences of HTO and H_2O between soil air and atmosphere. Ideal conditions are given, if soil moisture and density are homogeneous and if the profile of the specific activity of the soil moisture is strongly exponential. The effect of deviation from the ideal conditions is about 30% in the case of the measured profiles of the field experiments. The scaling length defined above, characterizes the vertical HTO distribution within the soil and hence represents a measure for the availability of HTO for reemission

4. Summary

To some extend the HTO reemission is coupled to H_2O reemission (evaporation), namely if the driving forces for the water movement in the soil and for the transformation from the liquid to the vapour phase at the soil atmosphere interface are considered. The potential reemission of HTO and H_2O is depending on the same soil physical and micro-meteorological parameters. But the effective reemission of both types of molecules are governed by their relative abundances in soil moisture and air humidity and, therefore, usually different from one another.

5. Publications

Täschner, M., Bunnenberg, C.:
Tritium Reemission from Soil in a Wind-Tunnel.
NIR-Jahresbericht 1992 (annual report), in press.

6. Main contributors to the project

Dr. M. Täschner, Mrs. G. Erb- Bunnenberg, Mr. R. Sachse.

Head of project 2: Dr. Y. Belot

II. Objectives for the reporting period

The objectives of the overall contract period (1.09.92 to 1.05.94) were: (i) to study the reemission of tritium from ground-level sources and from soils by small-scale field experiments; (ii) to compare the field data to those obtained from laboratory experiments; (iii) try to improve the existing theoretical models.

During the reporting period (1.09.92 to 1.09.93) our efforts were first focused on the dispersion of tritium into the atmosphere from a ground-level source. First experiments were carried out to determine the vertical profiles of atmospheric concentration at short distances of a point source placed on the soil. The first series of profiles obtained under unstable weather conditions were compared to predictions based on realistic wind speed and eddy diffusivity profiles. Further work is still to be done to study the form of the concentration profiles under neutral or stable conditions.

Other experiments were devoted to the reemission of tritiated water from small contaminated soil surfaces. These experiments aimed at determining the fraction of deposited tritium that is reemitted per unit time to the atmosphere in the hours following exposure. The data obtained are to be compared to predictions based on models of tritium movement within a multilayered soil profile and through the soil-atmosphere interface. This part of the study is still at its beginning and needs to be developed in order to validate and / or improve the existing models.

III. Progress achieved

1) Experimental methodology

The short-range dispersion of HTO from a small area source was studied under the conditions prevailing in the open air, by placing a vertical sampling grid perpendicular to the wind direction at a small distance downwind the source. The sampling grid is made of 5 horizontal tubes, each perforated with 14 equidistant holes. Each tube is closed at one end and connected at the other end to a water trap filled with molecular sieve. All the traps are connected to a vacuum manifold pumped at a sufficient flowrate to maintain a low pressure in the tubes and obtain that the sampling orifices function in the sonic mode with equal and constant individual flowrates. The tritium collected in each trap is thus representative of the space-averaged concentration along the corresponding horizontal perforated tube, allowing the determination of the cross-wind integrated concentration (C) profile. The tritium is extracted from the molecular sieve by heating the molecular sieve at 500°C, flowing a nitrogen stream through it and recovering the tritiated water in a condenser.

In parallel, the wind speed (U) is measured at each sampling level by using hot film anemometers allowing accurate measurement of the low wind speed encountered close to the ground. Furthermore, the temperature (Θ) is measured at the same levels with high precision thermistors shielded by two ventilated concentric cylinders. All the micrometeorological data are recorded at five seconds intervals for the whole duration of each experiment (0.25 to 0.5 hour). The wind speed and temperature time-averaged profiles are used to calculate the friction velocity U_*, the friction temperature Θ_* and the Monin-Obukhov lenght L that characterises the weather stability.

The total amount of tritium emitted from the source to the atmosphere per unit time during the sampling period is obtained by numerical integration of the product (C * U) over the total sampling height. Great care should be taken that the sampling grid be placed at small distance from the source to encompass the totality of the plume cross-section. If, in a particular experimental run, the horizontal wind direction fluctuates accidentally in a very wide angle and if a part of the plume then escapes from the grid without being sampled, the run should not be considered further. The grid should also be placed far downwind any obstacle to avoid any perturbation of the horizontal trajectory of the wind that could result in an upward movement of the plume over the grid. If these precautions are taken, the method allows the determination of reemission rates during small periods of time, of a quarter of an hour or so, with a reasonable accuracy for such field experiments.

2) Transport of tritium from a ground-level source into the atmosphere

The dispersion of tritium and more generally of any passive tracer from the soil into the atmosphere has been tentatively described by numeric integration of the Gaussian plume model over the surfaces of interest. Unfortunately, this basic model has been established for elevated sources and for the calculation of ground-level concentrations at relatively large distances from the sources (beyond 100 m). Also, we may have some doubt about the validity of such a model to calculate the concentrations in the atmosphere that result from short-range transport of a passive contaminant emitted at ground-level and diffusing in a thin layer of the atmosphere where gradients of wind speed and diffusivity are particularly important. In this work we began to determine experimentally the vertical profiles of concentrations due to a small surface area source that can be nearly assimilated to a point-source, and to compare the data obtained to those derived from the different models that have been proposed up to now.

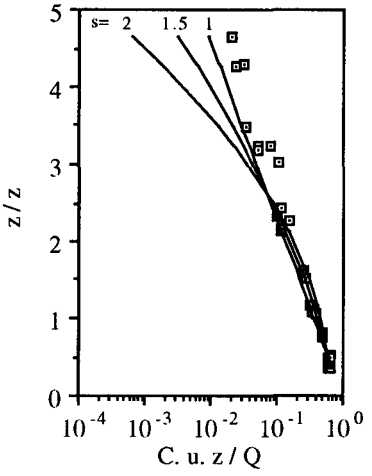


Fig.1 : Comparison between experimental and theoretical profiles of cross-wind integrated concentrations at distance x between 0.2 and 0 8 m and for a roughness lenght of about 0.01 m. The mean height of tracers particles is between 0.094 et 0.132 m. The weather was instable (stability class A) . The dots represent experimental data (Valduc 24.6.93) and the full lines represent theoretical forms of the concentration vertical profile.

A first experiment was performed on an approximately homogeneous field covered with short grass (z_0 = 0.01 m). The sources of HTO consisted of disks of blotting paper (backed with an impermeable plastic sheet) impregnated with 0.25 ml of a HTO solution placed at the center of the disk (about 1 MBq). 5 runs of 10 to 20 minutes duration each, were performed under unstable weather conditions. The Monin-Obukhov lenght was between -3.6 and -10.3 m, which corresponds to the Pasquill stability class A. The wind speed and crosswind-integrated concentration experimental profiles were used to calculate the mean height $\overline{z}$ of molecules that have travelled to a distance x, the mean horizontal velocity $\overline{u}$ of these molecules and the apparent emission rate Q. The distance x between the source and the sampling grid was in the range 0.2 - 0.8 m.

Figure 1 represents the experimental data (5 runs x 5 levels = 25 points) expressed in a non-dimensional form. On the same figure are plotted three theoretical profiles classically derived from the diffusion equation and based on realistic profiles of wind speed and eddy diffusivity near the ground. These theoretical profiles are represented by the Robert's equation:

$$C / Q = a / \overline{u}\,\overline{z}\, \exp [-b (z / \overline{z})^s]$$

where a, b and s are constant parameters depending on weather stability. The comparison between the experimental and theoretical profiles indicates that, in case of unstable weather, the concentration distribution is close to an exponential form (s=1, a=1, b=1), and very different from a Gaussian form (s=2, a = 0.64, b = 0.32). It appears also that the wind speed is to be

specified as the mean horizontal velocity of the diffusing tritiated water molecules and consequently as an implicit function of downwind distance. Further work is planned to determine the influence of weather stability on the form of the concentration profile.

3) Reemission of tritiated water from a contaminated soil surface

Field experiments were also carried out to determine the reemission of tritium in the open air from a small-area of undisturbed soil that has been classically labelled by deposition of HT in a small enclosure of 30-cm diameter placed onto the soil. The amount of tritium (Q) reemitted per unit time during a given period of reemission was determined by using the methodology described above that consists of sampling the plume, and integrating the product (C * U) over the total sampling height. Preliminary test experiments, envolving evaporation of a known amount of HTO from an artificial source placed onto the soil, indicated that the estimated emission rate is generally between 60 to 90 % of the actual emission rate, if the standard deviation of the horizontal wind direction is not greater than about 40°.

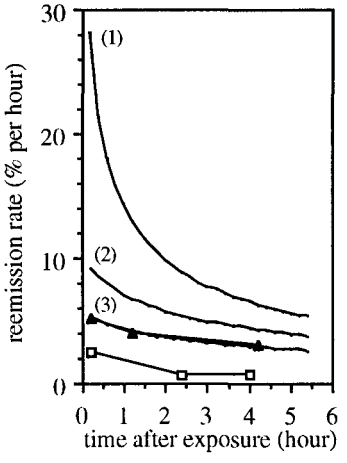


Fig.2: Comparison between theoretical predictions and experimental data. The theoretical curve (1) corrresponds to a soil of uniform moisture (10 %); the curves (2) and (3) correspond to a soil of the same moisture with a 2mm and 4mm-layer of dry soil on the surface. The black dots (Brennilis 5.8 92) and white dots (Cadarache 2 6.93) represent data from field experiments.

Six series of experiments were performed at three different locations, corresponding to different types of soil. The duration of soil exposure was about 0.7 hour; then the chamber was removed and a first determination was made of the reemission rate, defined as the percentage of tritium reemitted per unit time. The initial value of the reemission rate was between 2 and 5 % per hour. The values determined at greater time post-exposure were lower and lower as time elapsed as shown on Fig.2. Experiments are in preparation to study particularly the influence of the soil top-layer moisture on the initial reemission rate, because it appeared from comparison with theoretical predictions that the moisture at soil surface could be a critical parameter in the establishment of the tritium flux level (cf. next section). It will be also of a great interest to determine if the initial reemission rate differs when a soil is contaminated by HTO instead of HT.

The data obtained in the field experiments reported above were compared to predictions obtained from a code that calculates the deposition of HT and the subsequent reemission of HTO under realistic conditions. This code is based on the resolution of a series of linear differential equations that describe the exchange of HTO at the soil-atmosphere interface, and the transport of HTO inside the soil. Calculations were first made for a bare soil of uniform moisture distribution (10 %), exposed to HT during 10 minutes. The curve (1) in Fig.2 shows the values of the calculated reemission rate at different times post-exposure. The theoretical ree-

mission rate is 17 % and 12 % per hour at 1 and 2 hours post-exposure, these values tend to increase for greater soil moistures. The theoretical values are much higher than those determined in field experiments. This may be due to the non-uniform distribution of moisture in the soil top-layer. As soon as the rate of water evaporation exceeds the rate of flow of water to the soil surface, the evaporating surface retreats into the soil. This may result in a drop of the tritium reemission rate, because the need for the HTO vapour to diffuse to the soil surface lenghtens the effective vapour diffusion pathway, and hence increases its resistance to vapour flow. The curves (2) and (3) in Fig.2 show the theoretical reemission rates from a soil of 10 % moisture with a 2 mm and 4-mm layer of dry soil on the surface. These values were calculated by considering that the resistance of the soil-atmosphere interface to vapour diffusion includes a soil resistance in supplement of the aerodynamic resistance. This supplementary soil resistance was taken as $r_s = h_s / (\theta D)$, where h_s is the thickness of the dry layer, θ its void fraction and D the diffusivity of HTO vapour. It can be seen that the theoretical initial reemission rate thus obtained is relatively close to the low reemission rates determined in our experiments. Further experiments are nevertheless required to substantiate our hypothesis. These experiments should involve for instance the determination of initial reemission rates from the same soil with different moisture contents in the top layer.

IV. Main contributors:
H. Camus, S. Raviart and A. Charlier de Chily.

Head of project: Dr. Kim

II. Objectives for the reporting period

In the first period of the contract, an accurate analytical method will be further developed for the determination of tissue water tritium (TWT) and organically bound tritium (OBT) (exchangeable as well as non-exchangeable) in the biosystem, as discussed in our previous work [1-4]. This includes analysis and quantification of all discernable analytical effects, which can alter the original in vivo T/H ratios under consideration through sample preparation prior to tritium measurement. The method will be applied for the determination of the specific activity ratio of OBT and TWT (R value) of some representative biological samples.

The experimental R values as biological partition factors between the organics and tissue water of the biosystem will be verified for the dynamic equilibrium state between biotritium and environmental tritium. The R values will be measured as a function of growth time under constant HTO exposure in a climate chamber specially designed for the purpose.

In the second period of the contract, the analytical problems connected with the separation of free and bound water in soils will be investigated by analogy with the process applied for the biosystem. Distinction will be made between physisorbed tritium in capillary water and chemisorbed tritium associated with soil humus and mineral lattices. This necessitates a clear separation of the different tritium components in soils and requires analysis and quantification of possibly disturbing effects such as evaporation, solvation and adsorption isotope effects. Model substance/water systems will be compared with real soil/water systems.

III: Progress achieved including publications

Introduction

The radiotoxicity of tritium is increased by conversion from tissue water tritium (TWT) to organically bound tritium (OBT) which is retained much longer in the biological system. The OBT can be accumulated along the food chain by eventual enrichment processes resulting from isotope effects on metabolic enzyme reactions. Biological tritium conversion is quantified by a fractionation factor, expressed as the specific activity ratio (R) of OBT and TWT. Although a large number of R values have been reported in the literature, the great variation of the measurement data from 0.5 to 20 does not allow a reliable answer to the real extent of true biological tritium fractionation.

In the present study, outlined in detail elsewhere [5], all discernible factors which might affect the R value are analyzed critically and the conditions are established for determining the pertinent R value as a biological tritium fractionation factor. Under well defined conditions the R value is determined for barley (C_3 plant) and maize (C_4 plant). The two plant categories with distinct photosynthetic CO_2-fixation pathways, adapted to their different water use efficiency, are compared in order to estimate the natural variation of the biological tritium fractionation factors among different biosystems.

Results

Accurate determination of R values of biosystems

The total R value of a biosystem, defined as the specific activity ratio of total (exchangeable and non-exchangeable) OBT and TWT is inevitably altered through vaporization isotope effects occuring at the separation of the organic and water components. The tritium enriched residual water exchanges with the labile OBT, which remains enriched even after complete tissue water removal. The effect increases with decreasing temperature and has been quantified in our previous work [1-4]. The error of the total R value may increase up to + 400 % depending upon physical parameters, such as temperature of water vaporization, degree of water removal, water content of the original sample, etc. The minimum attainable error still amounts to about 15 % in the case that tissue water is completely removed (> 99.9 %) at high temperature, e. g. by azeotropic distillation at 70 °C. This method of water separation has been applied in this work. The analytical isotope effects have been mathematically corrected on the basis of experimentally determined input data for the chosen biosystems, namely barley and maize plants [4]. A similar correction can be performed only for the case of water separation by distillation at constant temperature, accompanied with a constant separation factor. The analytical isotope effects at freeze drying of the biosample, performed at changing temperature and system pressure are unpredictable and therefore not corrigible. Another precaution is a careful elimination of air humidity in contact with the sample in order to avoid isotope dilution by condensation or exchange reactions.

The stable R value of a biosytem, defined as the specific activity ratio of non-exchangeable OBT and TWT, requires the removal of exchangeable OBT through at least two washing procedures with tritium free water and correction for isotope dilution by wash water, physisorbed to the organics.

R value as a biological tritium fractionation factor

The R value of a biosystem does not always correspond to a biological tritium fractionation factor for the equilibrium state between the considered organic and water phases. The equilibrium state can be experimentally achieved by growing the biosystem under constant HTO exposure until constant R values are attained. If this condition is not fulfilled, the R value is a sum of two simultaneously occurring biological effects, namely natural tritium fractionation due to isotope effects on metabolic enzyme reactions, the parameter of interest, and different biokinetics of TWT and OBT. The latter effect reflects the fluctuations of the HTO exposure with a different time delay for the water and the organic components corresponding to their respective biological half lives. This source of error occurs easily if samples are taken from the natural environment.

Fig. 1 compares the accurate biological tritium fractionation factors (percent) for barley and maize. The OBT discrimination in barley (C_3) is higher than in maize (C_4). The mean difference of OBT_{total} and OBT_{stable} discrimination amounts to (13.3 ± 2.3) %. This is not surprising, since the two plants belong to different metabolic types. They have distinct biochemical and physical regulation mechanisms tending to an optimal compromise between the often contrary requirements of photosynthesis and water balance. The water loss by transpiration is e. g. limited in C_4 plants through closing of the stomata. The accompanying hindrance of CO_2 uptake is compensated by decreasing the CO_2 consuming photorespiration. It necessitates photosynthetic enzyme systems coupled with isotope effects which are different from that of C_3 plants. Contrary to the OBT, the TWT of barley has a higher specific activity (10.5 ± 0.7) % than that of maize. The difference seems to be related mainly with physical rather than chemical isotope effects. The higher transpiration rate of C_3 plants as compared with C_4 plants favors tritium enrichment in the tissue water of barley through the vapor pressure isotope effect. The slow transpiration rate of C_4 (desert) plants diminishes the influx

of water into plants by viscous flow. It facilitates the exchange reaction of hydrogen between tissue water and atmospheric water, which specific activity is 10 % lower than that of the nutrient solution at 20 °C [2].

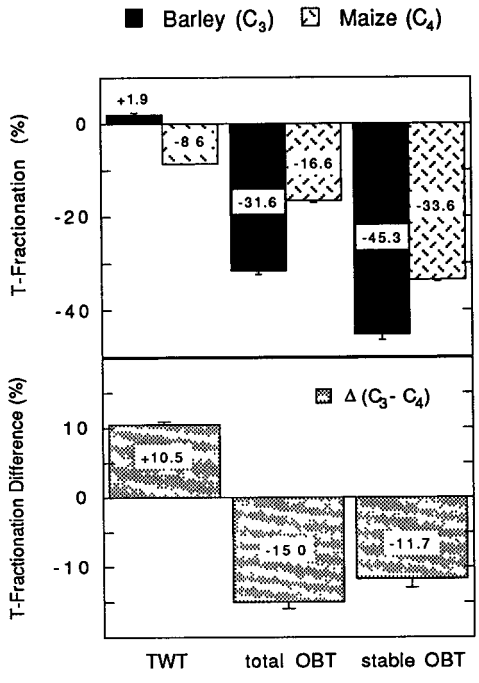


Figure 1 : Comparison of the biological tritium fractionation factors (%) of barley (C_3 plant) with those of maize (C_4 plant)

List of publications

[1] Kim M. A. (1988) Validation of tritium measurement in biological materials. *Fusion Technology* **14**, 1153.

[2] Baumgärtner F. and Kim M. A. (1990) Isotope effects in the equilibrium and non-equilibrium vaporization of tritiated water and ice. *Int. J. Appl. Rad. Isot.* **41**, 395.

[3] Kim M. A. and Baumgärtner F. (1991) Tritium fractionation in biological systems and in analytical procedures. *Radiochim. Acta* **54**, 121.

[4] Kim M. A., Baumgärtner F. and Schulz C. (1991) Accurate determination of tritium in biosystems. *Radiochim. Acta* **55**, 101.

[5] Kim M. A. and Baumgärtner F. (1993) Equilibrium and non-equilibrium partition of tritium between organics and tissue water of different biological systems. *Int. J. Appl. Rad. Isot.* submitted for publication.

Head of project 4: Prof.Dr. Dertinger

II. Objectives for the reporting period

The consideration of organically bound tritium (OBT) formation in crop plants after a contamination of the environment with tritium is essential for the calculation of the dose due to the ingestion pathway. The most important process of OBT formation is photosynthesis in green leaves. The quantity of OBT formation is mainly a function of light intensity and temperature during and after a tritium release. In the generative stage of development, OBT is translocated from the leaves to fruits and other storage organs.

The quantity of OBT formation and translocation is investigated by exposure of wheat and potato plants to atmospheric HTO under defined laboratory conditions. Potted plants of spring wheat should be exposed at different stages of development, and the resulting OBT concentration in the grains at the time of harvest should be analyzed. The relevant meteorological and plant physiological parameters are additionally recorded for laboratory conditions as well as for natural field conditions. Experimental results and different data sets from field measurements are used to calibrate a photosynthesis model which is newly developed.

In the next reporting period the accumulated samples from this year's exposure experiments will be analyzed for tissue free water tritium (TFWT) and OBT. The concentrations will be measured separately in the different plant compartments, as e.g. ears, stems, leaves and grains (tubers in case of potatoes) at the time immediately after exposure as well as some days after exposure, and at the time of harvest. After evaluation, these data can be used directly to calibrate the photosynthesis model, which is consisting of 11 plant compartments at the present stage of development

The data sets from the gas exchange measurements in the field during this year's vegetation period as well as growth parameters, e.g. leaf area index, dry matter of the grain etc. , will be evaluated and brought into a form in which they can be used together with the meteorological data as input to the computer code of the plant model. A calibration of the model for winter wheat shall be achieved with these data. For a subsequent validation of the model, by comparison of predicted concentrations by the model with measured concentrations in the grain at the time of harvest, it is anticipated to gain independent data sets during the vegetation period 1994

III. Progress achieved including publications

Translocation experiments 1992

In autumn '92 and winter '92/'93 we have analyzed the samples obtained from the exposure experiments with wheat and potatoes which have been carried out in June/July '92. We used spring wheat in eight different stages of development, one experiment was under night conditions. With potatoes only one experiment under night conditions was possible in 1992. The duration of exposure to atmospheric HTO was 2 hours at 24°C, about 90% relative humidity, and 7000 lux light intensity for the day conditions and at 16°C and about 90% relative humidity for the night conditions. The maximum HTO-concentration in the exposure box was between 2 and 6 kBq/l of air (120 -350 kBq/ml of air humidity).

The concentration of tissue free water tritium (TFWT) in the green leaves of wheat after 2 hours of exposure was about 60% of the specific tritium concentration in air humidity under day conditions and only 10 % under night conditions Although stomata are closed at night, some HTO can enter the leaves. After 2 hours of exposure, 0,2% of the HTO in the whole plant was incorporated into non exchangeable OBT at day conditions and 0,1 % at night conditions. That means that also other processes than photosynthesis are able to convert HTO into OBT

To describe the OBT incorporation into wheat grains as a function of the development stage we used a simple approach not considering the various processes from assimilation in the leaves to translocation to fruits. The specific concentration of OBT in grain at harvest (Bq/g) is related to the specific concentration in TFWT (Bq/ml) immediately after exposure to HTO. The course of this translocation coefficient as a function of the time after anthesis is shown in figure 1. The incorporation of OBT into the grains increases at the beginning of the grain filling period (about 1w after anthesis) and reached a maximum at about 2w after anthesis. In subsequent period the translocation coefficient decreases because the incorporation of assimilates decreases.

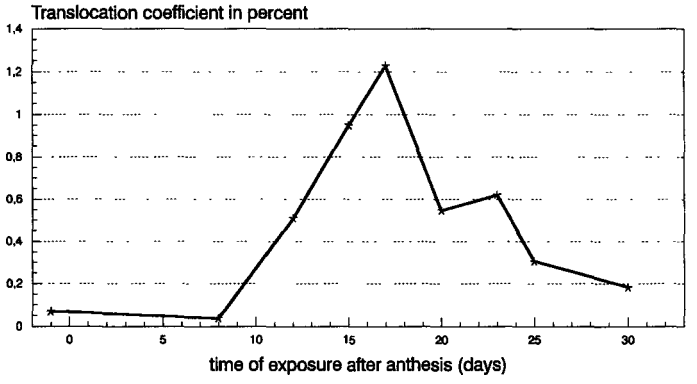


Figure 1: Translocation coefficients of OBT in spring wheat (percentage of the specific concentration of OBT in grains at harvest and the specific concentration of TFWT in leaves at exposure to HTO) as a function of the time of the exposure to atmospheric HTO under day conditions (TFWT = tissue free water tritium).

Translocation experiments 1993

In June/July 1993, additional exposure experiments have been performed. We exposed wheat at 8 different stages of development under day conditions and 2 different stages under night conditions. Two experiments with potatoes under day conditions and one under night conditions have been performed. The analyses of the samples is in work.

Model development

In co-operation with a modelling group in the KfK a specific plant model has been elaborated to predict formation, and translocation of OBT in the wheat plant and storage in the grain. The computer code is written in FORTRAN. Figure 2 gives an diagrammatic representation of the model in the actual stage of development.

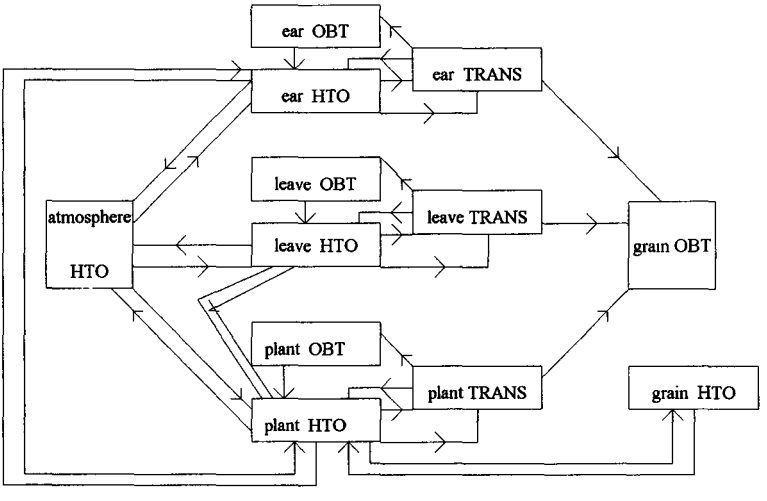


Figure 2: Compartment model for tritium in wheat (TRANS = fictive transport compartment in the different plant components)

Gas exchange measurements 1993

For model calibration and testing, sets of plant physiological as well as climatic data were collected in a field of winter wheat in a small valley (Weierbach) about 30 km away from KfK in north-east direction. The meteorological data were measured and recorded in this valley continuously by the KfK institute IMK since last year, and were placed at our's disposal.

Measurements of the rate of CO_2 assimilation , transpiration, and stomatal conductance were made using a gas exchange measuring system (Walz Meß- und Regeltechnik, Effeltrich, FRG). In time intervals of about 2 weeks during the development of the plants, typical diurnal changes of these parameters were measured at the upper leaf of one plant, as well as the maximum net photosynthesis rate at light saturation as a function of the leaf temperature. A preliminary evaluation of these data is in progress. They will now be brought into a format in which they can be used as input to the model. Figure 3 shows an example of the observed diurnal change of the net assimilation rate (respectively dissimilation in case of negative values) in the flag leaf of wheat and the corresponding photosynthetically active radiation (PAR) from May 12, 1993.

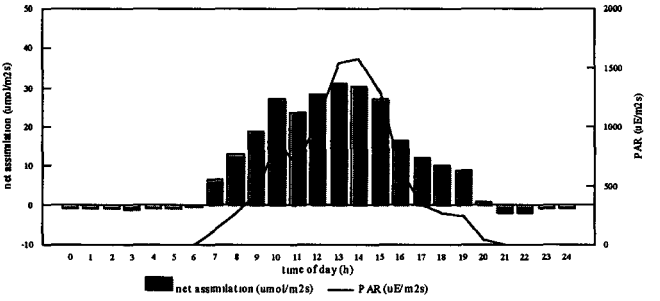


Figure 3: Diurnal trend of the measured rates of net assimilation in a flag leaf of winter wheat with the corresponding photosynthetically active radiation (PAR) on May 12, 1993

Acknowledgement:

These investigations have been performed by Silvia Diabaté, Jutta Müller, and Siegfried Strack. The technical assistance by Silke Kahmann is acknowledged. The model development was kindly supported by Wolfgang Raskob (D.T.I. Dr. Trippe Ingenieurgesellschaft mbH).

Publications:

• Diabaté, S., Strack, S., Studies on Translocation of tritiated assimilates into potatoes and wheat grains. In : Proceedings of the International Symposium on Radioecology, chemical speciation - hot particles , Znojmo, CSFR, October 12 - 16, 1992.

• Müller, J., Diabaté, S., Raskob, W., Strack, S., Untersuchungen zum Transport tritiierter Assimilate in Kartoffeln und Weizen, 25.Jahrestagung des Fachverbandes für Strahlenschutz, Sept. 1993, Proceedings in press

Progress Report

Contract: **F13P-CT920006** **Sector:A24**

Title: Transfer of radionuclides in animal production systems

1)	Howard	ITE
2)	Assimakopoulos	Univ. Ioannina
3)	Crout	Univ. Nottingham
4)	Mayes	Inst. Macaulay Land Use Research
5)	Voigt	GSF
6)	Vandecasteele	SCK/CEN, Mol
7)	Hove	AUN, Ås,
8)	Hinton	PSI

I. Summary of Project Global Objectives and Achievements

The studies conducted by the group under the previous CEC DGXIII-F-6 programme identified and quantified some of the most important factors responsible for determining the levels of radiocaesium in animal products. The current programme concentrates primarily on three mobile radionuclides: radiocaesium, radioiodine and radiostrontium.

The objectives of the two year programme are:
1. To provide improved information on the behaviour of radionuclides in animals to enhance the ability to predict contamination levels in animal derived foodstuffs.
2. To measure the true absorption coefficient (A_t) for radiostrontium and radioiodine with the aim of substituting this physiological parameter for the more mechanistic transfer factors currently used in predictive models.
3. To investigate factors affecting the plasma to tissue transfer of radiocaesium.
4. To improve the range of available countermeasures, especially for radiostrontium.
5. To identify important factors which may affect the behaviour of radioiodine in the environmental and agricultural conditions prevailing throughout Europe.
6. To investigate the characteristics of soil particles adhered onto vegetation surfaces which may be ingested by ruminants.

(i) Absorption

Initial studies to determine the A_t of radiostrontium and radioiodine have been conducted emphasis was placed on any potential adaptations necessary to the methodology when considering these two isotopes [01,04]. For ionic radioiodine, A_t was estimated to be approximately 1.0. We also found that the ratios of intravenously and orally administered isotopes in the thyroid could be used to give reliable estimates of A_t.

A similar study has been conducted for radiostrontium but a completed data set is not yet available [01,04]. A range of forage types contaminated by ^{85}Sr have been fed to sheep in a separate study [06] for which analyses of samples are currently underway.

Estimates of A_t for radiocaesium in lactating goats have been made. Absorption was calculated to be complete, this contrasts to sheep in which A_t values are usually around 0.85 [07].

(ii) Metabolic Behaviour of Radiocaesium

The effect of varying levels of stable caesium (0.35 to 2000 mg/day) administered to lactating goats continuously for a period of 45 days has been shown to have no effect on the transfer of radiocaesium to either milk or meat [07].

(iii) The Effect of Environmental Factors

Varying ^{137}Cs levels in pasture vegetation grazed by dairy cattle in a rotational grazing regime were not reflected in the milk activities [05]. Under a continuous grazing regime there was an increase in ^{137}Cs in vegetation of June-July the reasons for which will be investigated in collaboration with other participants. A lysimeter study to determine the effect of different simulated grazing pressures on the transfer of a range of radiologically significant radioiosotopes to vegetation is ongoing [05].

Results from soil adhesion studies indicate that is necessary to determine the particle size of the tracer and the tracee when considering the adhesion of soil particles to vegetation. Failing to account for this relationship probably explains the unreasonably high mass loading estimates obtained during the previous programme [08]. A possible method of directly estimating the bioavailability for transfer to grazing animals of radiocaesium from vegetation contaminated with adherent soil has shown promising potential [01]. Seasonal variations in bioavailability were found in agreement with that which may be expected due to seasonal changes in soil adhesion.

The transfer of ^{90}Sr from a sandy soil collected from the 30km Chernobyl zone to sheep milk is being measured. An initial tentative value for the soil-to-milk ^{90}Sr transfer coefficient is $f_m = (1.2 \pm 0.2) \times 10^{-2}$ d kg^{-1}, which is similar to previous values for the transfer of ionic strontium to the milk of small ruminants [02].

(iv) Countermeasures

Six different chemical compounds have been tested in studies using lactating goats to determine their effectiveness in reducing the transfer of radiostrontium to milk [07]. Only zeolites were shown to have a significant effect. A wider range of zeolites, clinoptilolites and mordenites will be investigated in future studies.

The effectiveness of pillared layer clays (PILCs) manufactured from montmorillonite as radiocaesium binders has been studied in *in-vitro* tests [02]. The binding capacity was shown to be comparable to that of AFCF. An assessment of the materials capacity to bind radiostrontium will also be made and *in-vivo* experiments using lactating goats conducted in collaboration with 07.

(v) Modelling

Data obtained from the previous programme by 04 on the behaviour of radiocaesium in the digestive tract has been used to improve the ruminant radiocaesium model [03]. The model now gives estimates of the apparent and true absorption coefficients which more accurately reflect measured values.

Current approaches to modelling the transfer of radiostrontium in ruminants do not take into account the controlling influence of calcium requirement on strontium absorption. Based on a consideration of the possible influence of calcium a different approach has been developed [03] and as a result a range of experiments have been planned ([01],[03],[04]) which will provide the necessary data to develop a more realistic, physiologically-based model.

Head of Project 1: B.J. Howard

II. Objectives for the reporting period

1. Investigate the methodology of determining A_t for radioiodine and radiostrontium.
2. Following on from the results of the previous programme, investigate the use of *in-vitro* bioavailability techniques in soil adhesion studies.
3. Co-ordinate programme.
4. Supply Nottingham University with data for modelling the behaviour of radiocaesium in sheep.
5. Design experiments to supply data for Sr/Ca modelling to Nottingham University in collaboration with MLURI.
6. Design experiments to determine the influence of dietary goitrogens and environmental temperature on the transfer of radioiodine in collaboration with MLURI.

III. Objectives for the next reporting period

1. In collaboration with MLURI conduct a series of experiment to determine the effect of Ca status on radiostrontium transfer to goats. After analyses the data will be supplied to Nottingham University for the development of a model to describe the transfer of radiostrontium in ruminants.
2. In collaboration with MLURI conduct a series of experiments to determine the effect of dietary goitrogens and temperature on the transfer of radioiodine to lactating goats.
3. Continue the co-ordination of the programme.

IV. Progress achieved including publications

True absorption of radiostrontium and radioiodine

ITE/MLURI have been routinely using dual isotope techniques to determine the true absorption coefficient (A_t) of radiocaesium for a number of years. A number of studies within the current programme involve the estimation of A_t for dietary sources of radiostrontium and radioiodine. Initial studies have been conducted to determine if any changes to the methodology used previously are necessary for studies involving these isotopes. Results for radiostrontium can be found in the MLURI section of this report, those for radioiodine are presented here.

No fundamental problems were anticipated in adopting the methodology since radioiodine has two convenient isotopes (^{125}I and ^{131}I) and previous evidence suggested that orally and intravenously administered iodine would behave similarly in the blood.

A range of artificially prepared sources were fed to sixteen, 10 month old, female Scottish Blackface sheep which were housed in metabolism cages and divided into four experimental treatments. The primary aim of the experiment, funded by the UK Ministry of Agriculture, Fisheries and Food, was to investigate potential differences in the bioavailability of different dietary radioiodine

sources. However, we took the opportunity to conduct a more rigorous sampling procedure which enabled us to address the methodological questions relevant to the CEC programme.

The sources used were: ionic ^{125}I absorbed onto paper filters, designed for Gilson P5000 pipettes; an organic soil; a mineral soil; and *Lolium perenne* grown on turves contaminated with ^{125}I. Each source was administered, encapsulated within a gelatin capsule, by oral dosing twice daily for a period of 10 days. A continuous infusion of ^{131}I solution via a catheter into the jugular vein was started concurrent to the first oral dosing of the ^{125}I sources. Total urine and faeces outputs were collected daily. At the end of the study the animals were slaughtered and their thyroid glands removed. Unfortunately, some animals had to be slaughtered before day 10, on days 6 and 9, due to blocked intravenous catheters.

The preferred method of determining A_t in such studies (see below) relies on the establishment of a stable ratio between the two isotopes, in urine, milk or a relevant tissue. For radiocaesium an equilibrium in urine was established within 4 days. Figure 1 shows the change in the ratio of ^{125}I:^{131}I in the urine of lambs on the soil and ionic ^{125}I treatments (^{125}I was not detectable in the urine of animals administered contaminated *L. perenne*). It can be seen that the ratio plateaued for all the animals on approximately day 5. Therefore it appears that the A_t of radioiodine can be estimated by such methods in studies of around eight days duration.

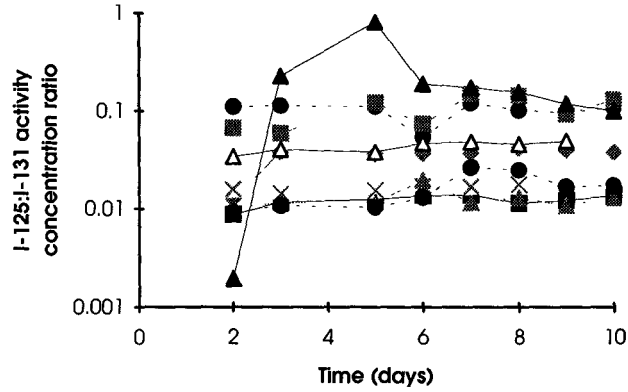


Figure 1. Changes in the ^{125}I:^{131}I ratio in urine for individual samples throughout the study.

Three approaches have been adopted to estimate the true absorption coefficient of ^{125}I from the dietary sources. These are referred to as A_{t1}, A_{t2} and A_{t3}:

1. A_{t1} = Blood plasma I-125 turnover rate (Bq/d) / I-125 intake (Bq/d)

where Blood plasma ^{125}I turnover rate (^{125}I BPT) =

Thyroid I-125 (Bq/kg) / Thyroid I-131 (Bq/kg) x I-131 infusion rate (Bq/d)

2. A_{t2} = Blood plasma I-125 turnover rate (Bq/d) / I-125 intake (Bq/d)

where ^{125}I BPT = Urine I-125 (Bq/kg) / Urine I-131 (Bq/kg)

3. A_{t3} = I-125 intake (Bq/d) - (I-125 faecal output (Bq/d) + I-125 EF (Bq/d)) / I-125 intake (Bq/d)

were I-125 EF is the endogenous faecal excretion of ^{125}I =

Urine I-125 (Bq/kg) / Urine I-131 (Bq/kg) x I-131 faecal output (Bq/d)

Values of A_t estimated using results for faeces and urine have been calculated as the average over the last four days of the study. Individual estimates of A_t are shown in Table 1. The various approaches used to calculate radioiodine A_t show reasonable agreement. There also appeared to be no effect of slaughter date on A_t values calculated using thyroid measurements. It therefore appears to be valid to use values calculated in this way for animals receiving contaminated *L. perenne* (for which it was not possible to estimate A_t by the other approaches used) in comparisons of availability from the various sources. The experimental results also suggest that it may be possible to *in-vivo* monitor the thyroids to determine the ^{125}I:^{131}I ratio in the thyroid to provide a more rapid estimation of A_t without the need for killing the animals.

Table 1. A comparison of A_t values for ^{125}I from the different dietary sources.

^{125}I Source	Animal Number	Day Slaughtered	A_{t1}	A_{t2}	A_{t3}
Ionic	1	10	1.00	1.13	0.93
	2	10	1.22	1.32	0.96
	3	10	1.66	1.33	0.92
	4	6	1.25	nd	nd
Organic soil	5	10	0.85	0.81	0.95
	6	9	1.13	nd	nd
	7	6	1.11	nd	nd
	8	10	0.80	0.75	0.92
Mineral soil	9	10	0.99	0.91	0.92
	10	10	0.86	1.06	0.95
	11	10	0.67	0.79	0.69
	12	10	0.71	1.26	0.96
L. perenne	13	9	0.61	nd	nd
	14	6	0.73	nd	nd
	15	6	0.75	nd	nd

nd = not determined

A method to predict seasonal changes associated with levels of adherent soil on the bioavailability of radiocaesium from pasture grass samples

In the previous programme a number of the collaborating laboratories conducted vegetation and soil sampling over a 12 month period from a range of different pasture types. The aim was to determine the importance of soil adhering to vegetation as a source of radiocaesium to grazing animals. Titanium was used as a marker of soil contamination of vegetation. However, for some months the results indicated that greater than 100 % of the radiocaesium in vegetation samples was due to soil contamination. It was concluded that this obvious overestimate was due to problems with the sampling protocol adopted. However, for most animal related studies the ultimate aim of such measurements is to be able to predict the likely level of contamination in the tissues of grazing animals. Therefore an alternative approach has been tested which utilises an *in-vitro* extraction technique (a 2 hour extraction with 0.1M CsCl followed by filtration through a 0.2μ membrane filter) to determine the bioavailability of radiocaesium from ingested sources for absorption in the animal gut developed by ITE/MLURI. Samples collected by ITE from an upland pasture in the UK during the previous programme have been used in this assessment .

Between 35 and 50 % of the ^{137}Cs associated with vegetation samples was extracted. This compares to a value of approximately 70 % extracted from fresh grass which was comparatively uncontaminated by soil during a previous study.

In January, March and April the amount of radiocaesium extracted fell to between 2 and 14 %. Initial results suggest a relationship between the Ti content of vegetation and the bioavailability of radiocaesium from vegetation indicating a reduced transfer to animals of radiocaesium from vegetation contaminated with adherent soil.

Data for modelling radiocaesium behaviour in sheep

Nottingham University has been supplied with data to model the transfer of radiocaesium to different aged sheep and from sources of differing bioavailability.

Publications

Beresford, N.A., Mayes, R.W., Crout, N.M.J., Howard, B.J. & Kanyar B. (*in press*) Dynamic behavior of ^{110m}Ag in sheep tissues. *Hlth. Phys.*

Crout, N.M.J., Beresford, N.A., & Howard, B.J. 1993. Does soil adhesion matter when predicting radiocaesium transfer to animals? *J. Environ. Radioact.*, **20,** 201-212.

Galer, A.M., Crout, N.M.J., Beresford, N.A., Howard, B.J., Mayes, R.W., Barnett, C.L., Eayres, H.F. & Lamb, C.S. 1993. Dynamic radiocaesium distribution in sheep: measurement and modelling. *J. Environ. Radioact.*, **20,** 35-48.

Hove, K., Strand, P., Voigt, G., Jones, B.-E.V., Howard, B.J., Segal, M.G., Pollaris, K. & Pearce, J. 1993. Countermeasures for reducing radioactive contamination of farm animal products. *Sci. Tot. Environ.*, **137.**

Howard, B.J. 1993. Management methods of reducing radionuclide contamination of animal food products in semi-natural ecosystems. *Sci. Tot. Environ.*, **137.**

Mayes, R.W., Beresford, N.A., Lamb, C.S., Barnett, C.L., Howard, B.J., Jones, B.-E.V., Eriksson, O. Hove, K., Pedersen, Ø. & Staines, B.W. 1993. Novel approaches to the estimation of intake and bioavailability of radiocaesium in ruminants grazing forested areas. *Sci. Tot. Environ.*

Voigt, G., Howard, B.J., Vandecasteele, C.M., Mayes, R.W., Belli, M., Sansone, U., Stakelum, G., Colgan, P.A., Assimakopoulos, N.M.J., Crout, B.E-V. Jones & Hove, K. *(in press)* Factors affecting radiocaesium transfer to ruminants. In: *Proc. Ann. 25th Meet. Fachverband fuer Strahlenschutz*, 28-30 Sep 1993, Rügen, Germany.

Wilkins, B.T., Howard, B.J, Desmet, G.M., Alexakhin, R.M. & Maubert, H. 1993. Strategies for the deployment of agricultural countermeasures. *Sci. Tot. Environ.*, **137.**

Head of Project 2: P.A. Assimakopoulos

II. Objectives for the reporting period

1. Measurement of the transfer coefficient for radiostrontium to sheep's milk due to soil ingestion.
2. Investigation of the effectiveness of modified smectites as caesium and strontium binders.
3. Further experimental investigation of the NPL animal model with regard to its applicability to a wide range of ruminants through a unique set of transfer parameters.

III. Objectives for the next reporting period

1. The analysis of strontium in the milk data from three more sheep will be determined in order to improve the accuracy on the value of the soil-to-milk transfer coefficient.
2. Further *in vitro* experiments will be performed with PILCs to determine their effectiveness as Cs and Sr binders. The effectiveness of this materials will be investigated in an environment of competing cations and a simulated rumen fluid.
3. An experiment involving four cows and five goats will be performed for the purposes of objective II.3 above.

IV. Progress achieved including publications

The following is a summary of the activities at the Nuclear Physics Laboratory (NPL), within the research programme between 1 September, 1992 and 31 August, 1993.

Soil-to-milk transfer coefficient for Sr-90 (Assimakopoulos, Pakou, Stamoulis)
In a previous experiment soil ingestion as a source of radiocaesium contamination to ruminants was studied by measuring the transfer coefficient to sheep milk. Eight lactating ewes, housed in individual metabolism cages, were used. Seven grams per day of heavily contaminated sandy topsoil, collected in 1990 from the Chernobyl area, were administered orally to the animals for a period of one week. The daily dose intake in ^{137}Cs was 1835 Bq d^{-1}. During this contamination period, daily milk production and excreta output were measured. The ewes were monitored for an additional seven day decontamination period, while they fed on uncontaminated feed. Transfer coefficients were obtained through a best fit (minimum χ^2) of the data to predictions of a linear compartment model. The values obtained were $f_m(Cs) = (2.6 \pm 0.7) \times 10^{-2}$ and $f_u(Cs) = (5 \pm 2) \times 10^{-2}$ d kg^{-1} for radiocaesium transport to milk and urine, respectively. These results suggest that soil ingestion can be a major source of radiocontamination for sheep and other free-grazing ruminants.

The samples collected in this experiment were further analysed during the reporting period with regard to radiostrontium concentrations. Strontium-90 concentration in the soil was measured as 3031 ± 189 Bq kg^{-1}, which resulted in a daily dose intake of 433 ± 27 Bq d^{-1}. The analysis of milk samples from two of

the ewes has been completed and the data have been fitted to the NPL animal model. A tentative value for the soil-to-milk ^{90}Sr transfer coefficient obtained from the analysis so far is $f_m(Sr) = (1.2 \pm 0.2) \times 10^{-2}$ d kg^{-1}. This value is close to that obtained for ionic strontium administered to the animals.

The use of Pillared Layered Clays as Cs and Sr adsorbers (Assimakopoulos, Gangas, Pakou, Karamanis)
A wide class of natural materials, such as various kinds of clays (bentonite, smectites, micas, etc.) or industrial products, such as Ammonium Ferric Cyano-Ferrate (AFCF), have been tried in the past as countermeasures against radiocaesium contamination. However, these materials usually perform very poorly with regard to trapping bivalent cations such as strontium and are thus unable to perform as a general antidote against fallout contamination.

During the reporting period we have been investigating the trapping of both caesium and strontium by Pillared Layered Clays (PILC), a novel class of materials derived from swelling clays. These materials are cation exchangers for mono- as well as for multivalent cations.

PILCs are usually made from Montmorillonite, a clay mineral found in Bentonite ore. Montmorillonite is a layered clay with a cation exchange capacity (cec) of about 0.8 mEq/g. The exchangeable cations in this material are positioned in the space between successive clay layers. In pure water these layers disassociate to form a clay colloid, whereas in water of about 0.1 N or more, due to the presence of cations in the solution, the layers collapse and remain attached to each other. Thus in an environment like the rumen fluid the cation exchangeable positions in the clay interlayer space are not easily accessible. This makes bentonite much less effective than AFCF with regard to caesium trapping. The situation may be drastically improved by propping the clay layers apart by nano-size pillars of metal oxide and thus creating a network of pores in the interlayer space. The pores are about 1 nanometre high and extend from a few to about 100 nanometres in the two other dimensions. This pillared structure makes the exchangeable cation positions more easily accessible. Indeed the interlayer space in the modified clay presents a specific surface area (ssa) of a few hundred m^2/g, i.e. a ssa more that ten times that of the untreated montmorillonite. In addition the new structure is stable with respect to ionic concentration or acidity of the aquatic environment.

Two types of PILCs with aluminium oxide pillars, supplied by STRATON, hi-tech, Ltd., a Greek SME which produces these materials, were tested during the reporting period.

1. Product ATHENA, with an interlayer distance from 0.2 to 1 nm and a ssa of 130 m^2/g.
2. Product AZA, with interlayer distance of 1 nm and ssa of 220 m^2/g.

The kinematics of Cs - PILC exchange process were investigated in four series of experiments by submerging samples of PILC, sealed in dialysis tube, into a solution of radiocaesium. The rate of caesium uptake was determined by periodically removing the PILC and measuring the remaining solution activity. In particular the following experiments were carried out:

1. The two types of PILCs were compared to each other. They were found to adsorb Cs with an exponential behaviour and essentially the same relaxation time and capacity per gram of adsorbent.

2. The dependence of caesium adsorption on the PILC mass was studied by employing 150, 100, 60, 30 and 10 mg of ATHENA with 200 ml solution of ^{137}Cs concentration 1.48×10^{-11} mole/ml and pH around 6. After 100 hours the amount of caesium removed by the PILC was 99 %, 89 %, 84 %, 66 % and 40 % respectively. Thus the minimum PILC mass required for the removal of a predetermined percentage, say 90 %, of the initial Cs activity in a given solution was determined.

3. The PILCs' selectivity for caesium in competition with other cations (Na and K) was investigated by adding sodium and potassium to the solution. It was found that in the presence of these cations the adsorptive power of the PILC for caesium increased.

4. The product ATHENA was compared to AFCF with regard to caesium uptake under various conditions. In the presence of competitive cations and for the concentrations employed, the adsorptive capacity of PILCs is comparable to that of AFCF. In view of AFCF's poor performance for multivalent cations, this is a very encouraging finding.

A new series of similar experiments with ^{85}Sr solutions is scheduled to start in October 1993.

Plasma-tissue transfer of Cs in ruminants (Assimakopoulos, Pakou, Ioannides, Aslanoglou, Karamanis, Stamoulis)

The relationship:

$$f(k) = a(k)f(blood)$$

in which f(blood) is the feed-to-blood transfer coefficient, f(k) the transfer coefficient to a given compartment of the animal's body (soft tissue, lung, liver, etc.) and a(k) a constant, is predicted by our (Health Physics 61:245-253,1991) animal model. An experiment to verify this relationship was scheduled to begin on 1 February, 1993. To this end we contacted the Russian company INTERRADIOECO, based at Chernobyl, who promised to send us 7,000 kg of contaminated grass for a total price of US$ 2,000 by December 1992. A contract for this was signed between Professor Assimakopoulos and Mr Grebenyuk, representing INTERRADIOECO, on 15 November, 1992, at Znojmo, Czechoslovakia. On that occasion Mr Grebenyuk demanded and received an

advance of US$ 300. Unfortunately, in spite of numerous fax's and letters, Mr Grebenyuk (and the $ 300) have since disappeared.

Our plans are now to carry out this experiment with ionic caesium. To this end we have ordered a 162 μCi ^{134}Cs source, expected at Ioannina next September. The source will be used to construct several hundred pills in the form of cigarette filters encapsulated in gelatin. The pills, representing a dose of about 2000 Bq will be fed daily to 4 cows and 6 goats until the animals reach a state of equilibrium.

Publications

Assimakopolous, P.A. Ioannides, K.G., Karamanis, D.T. Pakou, A.A. Stamoulis, K.C., Mantzios, A.G. and Nikolaou, E. Radiocaesium transfer to sheep's milk as a result of soil ingestion, *Science of the Total Environment.* (in press)

Assimakopolous, P.A. Ioannides, K.G., Karamanis, D.T. Pakou, A.A. Stamoulis, K.C., Mantzios, A.G. and Nikolaou, E. Variation of the transfer coefficient for radiocaesium transport to sheep's milk during a complete lactation period, *Journal of Environmental Radioactivity.* (in press)

Assimakopolous, P.A. Ioannides, K.G., Pakou, A.A. Mantzios and Pappas C.P., Transport of radiocaesium from a sheep's diet to its tissues, *Science of the Total Environment.* (in press)

Head of Project 3: N.M.J. Crout

II. Objectives for the reporting period

1. To extend the existing sheep Cs model to consider absorption and secretion in the gut more realistically.
2. To develop of a prototype ruminant Sr model.
3. To further consider the factors affecting the dynamics of Cs in grazed swards.

III. Objectives for the next reporting period

1. To incorporate Sr data collected by ITE/MLURI (as it becomes available) into the prototype Sr model.
2. Develop a prototype iodine model for ruminants.
3. To test the radiocaesium models against further experimental data.

IV. Progress to date (including publications)

Sheep Cs Model

A model of Cs dynamics in sheep was developed during an earlier phase of this programme (Galer et al 1993) and has proved a useful research tool. Whilst quite successfully predicting tissue dynamics, limitations of the model have become apparent in relation to gut absorption. The model suggests little difference between 'true' and 'apparent' absorption coefficients whereas it has been shown experimentally that large differences exist. The model only allows limited recycling of Cs within the gut resulting in a pattern of absorption quite different from that observed. This could limit its usefulness for studies involving Cs binders such as AFCF.

Some improvements to the original Galer model have been made, resulting in a new variant (ANNE4). This model gives a true absorption figure closer, but still significantly lower, than that observed experimentally. A similar pattern of radiocaesium distribution within the body is maintained.

Data collected by MLURI in the previous phase of this project has been used to develop this model further, resulting in a new variant (GUT2). This model has an A_t value similar to those observed.

Table 1 gives several radiocaesium transfer characteristics predicted by the 3 models for sheep

TABLE 1.

	Literature	GALER	ANNE4	GUT2
F_f (d kg^{-1})	0.35	0.39	0.44	0.69
$T_{1/2}$ (d)	17	12	11	11
A_t	0.85	0.41	0.63	0.92
A_a	0.65	0.41	0.46	0.74

The models have been tested against several independent sets of data. The agreement overall for oral doses is encouraging. However there appear to be problems with IV doses of radiocaesium. Obviously this particular route is not important in radiation protection terms and has only been used for experimental purposes, nevertheless the model should be able to correctly predict the distribution of radiocaesium within the animal.

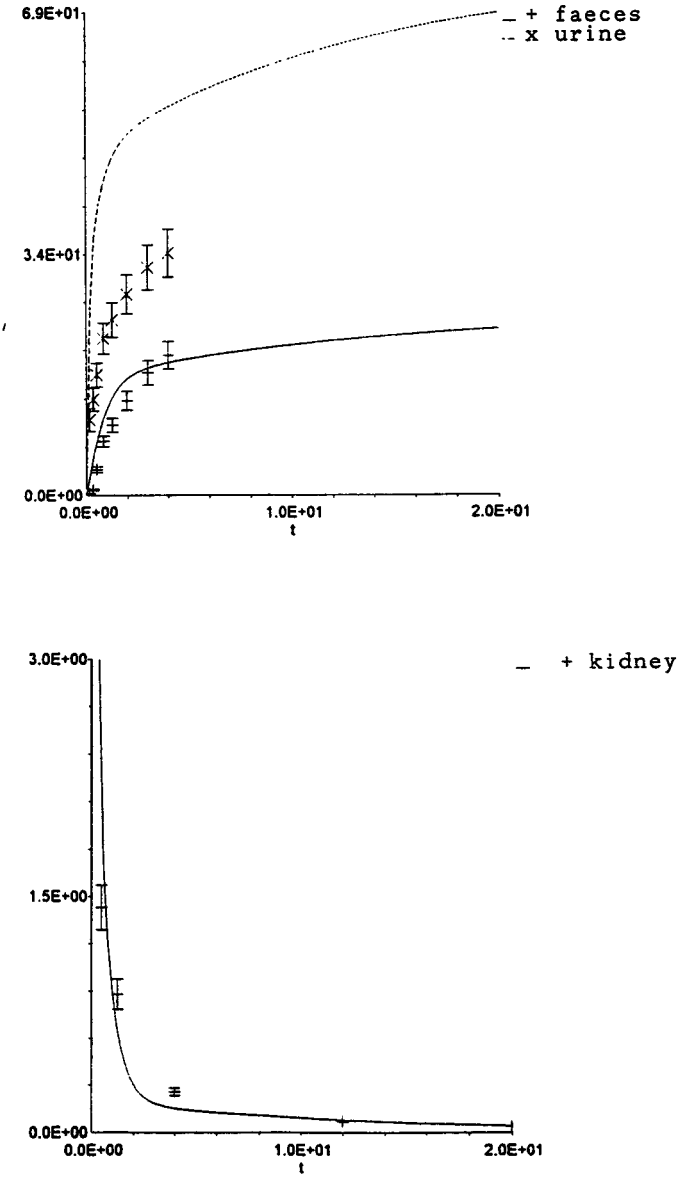
In particular comparing the model to single IV doses of radiocaesium there is a major disagreement between the predicted (using ANNE4) and observed excretion of radiocaesium via urine (see fig. 1), although the excretion to faeces is well modelled. The model also agrees well with other tissues measured, for example the kidney (see fig. 1).

From further analysis it has been realised that given the data used for the test this level of disagreement is inevitable at some point. The model was run assuming a body burden of 1.0 Bq of radiocaesium. The solution of the differential equations is numerically very accurate and no radiocaesium leaves the system, therefore throughout the simulation the model will 'contain' 1 Bq of radiocaesium. This is not the case with the experimental data. On three occasions it is possible to calculate a full inventory of radiocaesium within the animals, from both the oral and IV does. These values are given in table 2. Clearly there is a problem with the IV dose of radiocaesium as some of it is unaccounted for in the data. The reasons for this are unclear. The model cannot account for this 'missing' radiocaesium and it manifests itself by the large disagreement between predicted and measured urine output. Given the good agreement with the other compartments it may be that there was some error in the measurement of urine activity. However this is speculation at present.

TABLE 2.

Time (d)	Oral Dose Recovered (%)	IV Dose Recovered (%)
0.5	99	59
1	101	65
4	102	72

Figure 1. (top) A comparison of predicted and measured outputs of radiocaesium in faeces and urine; (bottom) a comparison of predicted and measured radiocaesium activity concentrations in the kidney.



Sr Model

There are a number of Sr models already available in the literature, for example the NRPB cow model (Simmonds 1985 (NRPB-M121)). The use of these models has been carefully considered within the programme with two conclusions:-

1. Existing models do not consider the controlling influence of Ca requirement on Sr absorption within the gut. Absorption of Ca by the animal varies in response to its requirement, and it is probable that this will dictate Sr absorption. Models with fixed rate coefficients, such as the NRPB model, will therefore only apply at one Ca requirement level.
2. Given the homeostatically controlled concentration of Ca within animals and its similarity to Sr there is little reason to doubt the validity of the existing models, once Sr has been absorbed from the gut.

On this basis a Sr model has been developed which extends the existing NRPB models to include the effect of Ca requirement on Sr absorption. This includes a number of parameters which are presently unknown, for example the discrimination between Ca and Sr and at the various points of transfer. Experimental work by MLURI/ITE has been designed to obtain the necessary data.

Grazing Effects on Cs Dynamics of Swards

Earlier work with the RUINS soil-plant Cs model suggested that variation in grazing intensity could affect Cs concentrations in vegetation, and thereby in animals. In the model this effect is due to changing the balance of vegetation loss from the sward due to senescence and grazing. During senescence Cs is assumed to be partly translocated to new growth. This is based upon ecological data for potassium. Independent field studies by Salt & Mayes (1991) observed such differences although the mechanism was not identified.

A literature review has been undertaken to improve the theoretical basis of the model and collaborative links maintained with University of Stirling (Carol Salt) who is planning further experimental studies under controlled conditions.

From the general literature on nutrient mobility within plants it seems likely that the grazing effect on Cs concentration will be dependent on both species and plant nutritional status.

Publications

These papers relate to studies conducted earlier in the programme. Further papers describing current work are in preparation.

Crout, N.M.J., Beresford, N.A., & Howard, B.J. (1993). Does soil adhesion matter when predicting radiocaesium transfer to animals? J. Environ. Rad. **20**, 201-212.

Galer, A.M., Crout, N.M.J., Beresford, N.A., Howard, B.J., Mayes, R.W., Barnett, C.L., Eayres, H. & Lamb, C.S. (1993). Dynamic radiocaesium distribution in sheep: measurement and modelling. J. Environ. Rad. **20**, 35-48

Beresford, N.A. , Mayes, R.W., Crout N.M.J., Howard, B.J. & Kanyar, B. (In press)Dynamic behaviour of Ag-110m in sheep tissues. Health Physics.

Voigt, G., Howard, B.J., Vandecasteele, C.M., Mayes, R.W., Belli, M., Sansone, U., Stakelum, G., Colgan, P.A., Assimakopoulos, N.M.J., Crout, B.E-V. Jones & Hove, K. (In press) Factors affecting radiocaesium transfer to ruminants. In: Proc. Ann. 25th Meet. Fachverband fuer Strahlenschutz, 28-30 Sep 1993, Rügen, Germany.

Head of Project 4: R.W. Mayes

II. Objectives for the reporting period

1. To carry out, in collaboration with ITE, studies with sheep to evaluate methods of estimating the true absorption coefficient for radiostrontium, radioiodine and calcium.
2. To develop analytical protocols to allow analyses for ^{45}Ca, ^{89}Sr and ^{90}Sr in samples of faeces and urine which also contain ^{125}I, ^{131}I, and ^{85}Sr.
3. Plan experiments to investigate the effects of calcium status on the behaviour of radiostrontium in lactating goats, to be conducted in collaboration with ITE.
4. Plan experiments, in collaboration with ITE, to examine the effects of dietary goitrogens and environmental temperature on transfer of radioiodine to the milk and thyroid of lactating goats.

III. Objectives for the next reporting period

1. To complete analyses of faeces and urine for ^{45}Ca, ^{89}Sr and ^{90}Sr.
2. Conduct experiments to investigate the effects of calcium status on the behaviour of radiostrontium in lactating goats, to be conducted in collaboration with ITE.
3. Carry out experiments, in collaboration with ITE, to examine the effects of dietary goitrogens and environmental temperature on transfer of radioiodine to the milk and thyroid of lactating goats.

IV. Progress achieved including publications

True absorption of radiostrontium and radiojodine

In the ITE section of this report an experiment was described which was designed to estimate the true absorption coefficient (A_t) of radioiodine in sheep, for various dietary sources.

The objectives of that experiment were equally applicable to radiostrontium. Three isotopes of radiostrontium exist which could be used in true absorption experiments - ^{85}Sr, ^{89}Sr and ^{90}Sr. Options are thus available to allow estimates of A_t for radiostrontium from different sources. Because of the close relationship between the metabolism of strontium and calcium concurrent measurements to determine A_t for dietary calcium were planned.

Since it is feasible to analyse samples for radiostrontium and calcium in the presence of radioiodine the investigation to determine A_t for radiostrontium from different dietary sources was conducted at the same time as the radioiodine study in the same animals. The radiostrontium sources tested, in separate groups of four sheep, were: a) *Lolium perenne* (^{85}Sr), contaminated by root uptake and ionic ^{90}Sr absorbed into paper filters (safety filters for Gilson Pipetman P5000), b) an organic soil (^{85}Sr), c) a mineral soil (^{85}Sr), and d) Clinoptilolite (^{85}Sr). The *Lolium perenne* and the two soils were same materials which were contaminated with ^{125}I in the radioiodine study. The clinoptilolite was obtained from BNFL, Sellafield, Cumbria UK and powdered prior to contamination. For convenience of administration all sources were dosed orally in gelatin capsules twice daily for 10 days. A continuous intravenous infusion of ^{89}Sr was

administered concurrent with the oral dosing period. For the purpose of estimating A_t for dietary calcium an intravenous infusion of ^{45}Ca was also administered at the same time. Total collections of faeces and urine were carried out over the administration period.

Since ^{85}Sr is a gamma emitter it was possible to obtain analyses, with little sample preparation, concurrent with the radioiodine analyses in the same samples. Thus ^{85}Sr analyses are complete. Involved chemical preparations (outlined below) are required for the analysis of ^{45}Ca, ^{89}Sr and ^{90}Sr; these analyses have not yet been completed.

Results from the [^{85}Sr]-sources can be expressed as the apparent absorption coefficient (A_a), which does not take into account the endogenous faecal excretion of ^{85}Sr:

$$A_a = \frac{\text{Sr-85 intake (Bq/d) - Sr-85 faecal output (Bq/d)}}{^{85}\text{Sr intake (Bq/d)}}$$

Results for A_a, estimated over the last four days of the study, are summarised in Table 1.

Table 1. A comparison of Aa values for ^{85}Sr from various dietary sources.

Source	A_a
*_L. perenne_	0.04
**Organic soil	0.06±0.040
Mineral soil	0.14±0.081
Clinoptilolite	-0.24±0.093

*One animal
**Two animals

Whilst A_a values can never be larger than A_t the low values obtained for A_a in sheep suggest a low bioavailability for all the sources studied. The mean negative values obtained for the clinoptilolite (indicating a faecal excretion rate in excess of the intake over the relevant collection period) suggest a particularly low bioavailability. Results for ^{89}Sr are required before more definitive conclusions can be drawn regarding comparisons in bioavailability between sources.

Development of analytical protocols for analysis of Ca-45, Sr-85 and Sr-90 in samples of faeces and urine

The experiment described above required the analysis, in the same samples, the analysis of ^{45}Ca and ^{89}Sr in faeces and urine. In one treatment, additionally, ^{90}Sr analysis was required. Allowance has also to be made for the presence of ^{85}Sr in the samples (analysed by gamma spectroscopy) and possible

contamination by isotopes of radioiodine. Preparation methods are being developed to enable these analyses to be carried out.

In general there are eight stages of analysis:

(i) Drying, (ii) ashing, (iii) dissolution of ash, (iv) coprecipitation of Ca and Sr (v) centrifugation or filtration and washing of precipitate (vi) dissolution of precipitate, (vii) counting for ^{89}Sr in aqueous solution (Cerenkov), (viii) concentration of solution, addition of scintillation fluid and counting for ^{45}Ca by liquid scintillation analysis.

Prior to ashing, carrier Ca and Sr and KNO_3 (oxidant) are added to urine. It is hoped that radiostrontium can be effectively removed from insoluble ash; otherwise HF digestion of faeces will be necessary. Standard solutions of each radionuclide will be used to evaluate efficiencies and quenching and the contribution ^{89}Sr makes to the ^{45}Ca counting conditions. Scintillations arising from ^{85}Sr will be taken into account in the same way, with reference to the activity determined by gamma spectrometry. For samples containing ^{90}Sr strontium and calcium will be separated by passage of the purified solution through Sr.Spec columns which bind to Sr ions when in nitric acid solutions.

Publications

Beresford, N.A., Mayes, R.W., Crout, N.M.J., Howard, B.J. & Kanyar B. (*in press*) Dynamic behavior of ^{110m}Ag in sheep tissues. *Hlth. Phys.*

Galer, A.M., Crout, N.M.J., Beresford, N.A., Howard, B.J., Mayes, R.W., Barnett, C.L., Eayres, H.F. & Lamb, C.S. 1993. Dynamic radiocaesium distribution in sheep: measurement and modelling. *J. Environ. Radioact.*, **20,** 35-48.

Mayes, R.W., Beresford, N.A., Lamb, C.S., Barnett, C.L., Howard, B.J., Jones, B.-E.V., Eriksson, O. Hove, K., Pedersen, Ø. & Staines, B.W. 1993. Novel approaches to the estimation of intake and bioavailability of radiocaesium in ruminants grazing forested areas. *Sci. Tot. Environ.*

Voigt, G., Howard, B.J., Vandecasteele, C.M., Mayes, R.W., Belli, M., Sansone, U., Stakelum, G., Colgan, P.A., Assimakopoulos, N.M.J., Crout, B.E-V. Jones & Hove, K. in press. Factors affecting radiocaesium transfer to ruminants. *In: Proc. Ann. 25th Meet. Fachverband fuer Strahlenschutz,* 28-30 Sep 1993, Rügen, Germany.

Head of project 5: G. Voigt

II. Objectives for the reporting period

1. True absorption of Iodine in lactating cows - Studies of the influence of stable iodine on the transfer of radioactive iodine into cow's milk
2. Field experiments to determine radiocaesium transfer from vegetation to milk under different grazing regimes
3. Simulation of grazing intensity in field plot experiments

III. Objectives for next period

1. Performance of the animal experiments for the influence of stable iodine on true absorption in dairy cows in coordination with MLURI/ITE.
2. Continuation of the field experiments and the simulation experiments concerning the influence of grazing intensity; evaluation and interpretation of data.

IV. Progress achieved including publications

Influence of stable iodine intake on cows of I-131 transfer to milk

These experiments are postponed until later in the contract period in order to benefit from the MLURI/ITE results gained with sheep and goats. The experimental procedures to determine the true absorption of iodine with the two radionuclides I-131 and I-125 are still being developed and evaluated at MLURI/ITE. As far as the experimental design stands experiments with lactating cows will be performed at GSF/Grub in order to study interactions of stable iodine and radioactive iodine on absorption and its transfer to milk. A publication by Howard & Voigt considering stable iodine as a countermeasure for radioiodine is in preparation. Intercalibration for activity determination of I-125 provided by ITE has been performed.

Effect of grazing intensity on radionuclide levels in animals

Field experiments to determine radiocaesium transfer from vegetation to milk under different grazing conditions

Two farms have been selected: they are located close to each other, and were presumably affected in the same way by the Chernobyl accident. Farm A (Kleinhöhenkirchen) carries out continuous grazing regime, farm B (Arnhofen) rotational grazing regime which is the more commonly used farming practice in Bavaria. The different pastures used for grazing dairy cows are marked in the map (A-D). The conditions such as number of animals and areas of the grazed pastures are given below. Soil characteristics were determined and found to be identical at the two places (pH 5, clay 16 %, silt 40 %, sand 44 %, cation exchange capacity 12.2 mval/100g). Soil activity concentrations have been determined for the different pastures of farm B and are currently being measured for farm A.

	Farm A	Farm B
Number of dairy cows	36	45
Area of pastures (m^2)	36 750	26 150 (A) 29 120 (B) 24 620 (C) 23 080 (D)

At farm A bulk milk samples (4 times 0.25 L) and vegetation samples (5 replicas, areas 0.0491 m^2) were taken once a week as soon as the animals were driven outdoors (start in June). Bulk milk samples (4 times 0.25 L probes resulting in one Litre milk samples to be measured) were initially taken at farm B daily (5.5.-22.5.93), and subsequently every other day (24.5. up to now;); also two replicates of vegetation samples over the pasture grazed at the moment with areas of 0.2332 m^2 were clipped by hand. Fresh weight of all grass samples was determined immediately after cutting, samples were oven-dried in the laboratory (70 ^{0}C) and weighed; all samples were measured by γ-spectrometry.

All dried vegetation samples are being kept for further analysis, i.e. soil adhesion measurements [08].

The activity concentrations of radiocaesium and ^{40}K in milk and vegetation in dependency of time are given for farm A and B in Figure 1 on a wet weight basis. The movement of the animals to the different pastures of farm B is given as well. Interpretation of these data is rather difficult for activity concentrations are very low and counting errors are sometimes high (about 20 %). However, no influence of the oscillating grass activities over time in one grazed pasture is reflected by the milk activities. The milk activities followed in 10 individual cows for 7 days in August (2^{nd} to 9^{th}) did not vary from the bulk milk activities (error within 5 %). Unfortunately the farmer could not be persuaded to allow us to determine herbage intake and composition using the alkane method.

Simulation of grazing intensity in field plot studies

Two different types of soils (one mineral and one organic soil) with relatively high Chernobyl-Cs-137 contamination (mineral soil: 86 Bq/kg fresh weight, organic soil: 130 Bq/kg fresh weight) were received via G. Lindner (Fachhochschule Ravensburg-Weingarten) in January 1993. Its soil characteristics were determined, and it was distributed into 15 aluminium frames (1 m^2) each giving a volume of about 70 Litre soil per area at the GSF experimental field. A weather station is located there and therefore weather data such as rainfall, radiation etc. are easily available. The soil plots were additionally contaminated artificially with a radionuclide mixture. The radionuclides were chosen with regard to their radioecological importance, commercial availability, their γ-energies (no interference with each other), and because of their short physical half-lives for radiation contamination limitation purposes. About 1 Litre of the radionuclide mixture containing:

Co-57 (63.7), Cr-51 (19.7), Cs-134 (65.0), Fe-59 (7.1), and Sr-85 (55.0 kBq/L)

was sprayed on the soil two days before seeding *Lolium perenne* (28.5.93), a pasture grass representative for Germany. Radionuclide activities were calculated and measured for each single area, activities per plot were recorded. Because of weather conditions (very dry and hot in June and July; very wet and cold from end of July on) grass growth was poor, especially on the organic soil. Therefore first cuts could be taken at the end of June from the mineral soil only. Further cuts were performed at 23^{th}, 30^{th} of July, 13^{th}, and 26^{th} of August. Three different sward heights (3, 6, and 9 cm) in five replicas each were clipped. Fresh weight was determined immediately, samples were oven dried at 70 ^{o}C, weighed and measured.

Because of the enormous amount of samples to measure and because of the very low ^{137}Cs activities, as well as the short half-lives of the artificial radionuclides in the vegetation samples we concentrate on ^{134}Cs, ^{57}Co, ^{51}Cr, ^{85}Sr and ^{59}Fe short term measurements. First results for the transfer of these nuclides in dependency of sward heights are available. All samples will be remeasured for ^{137}Cs later.

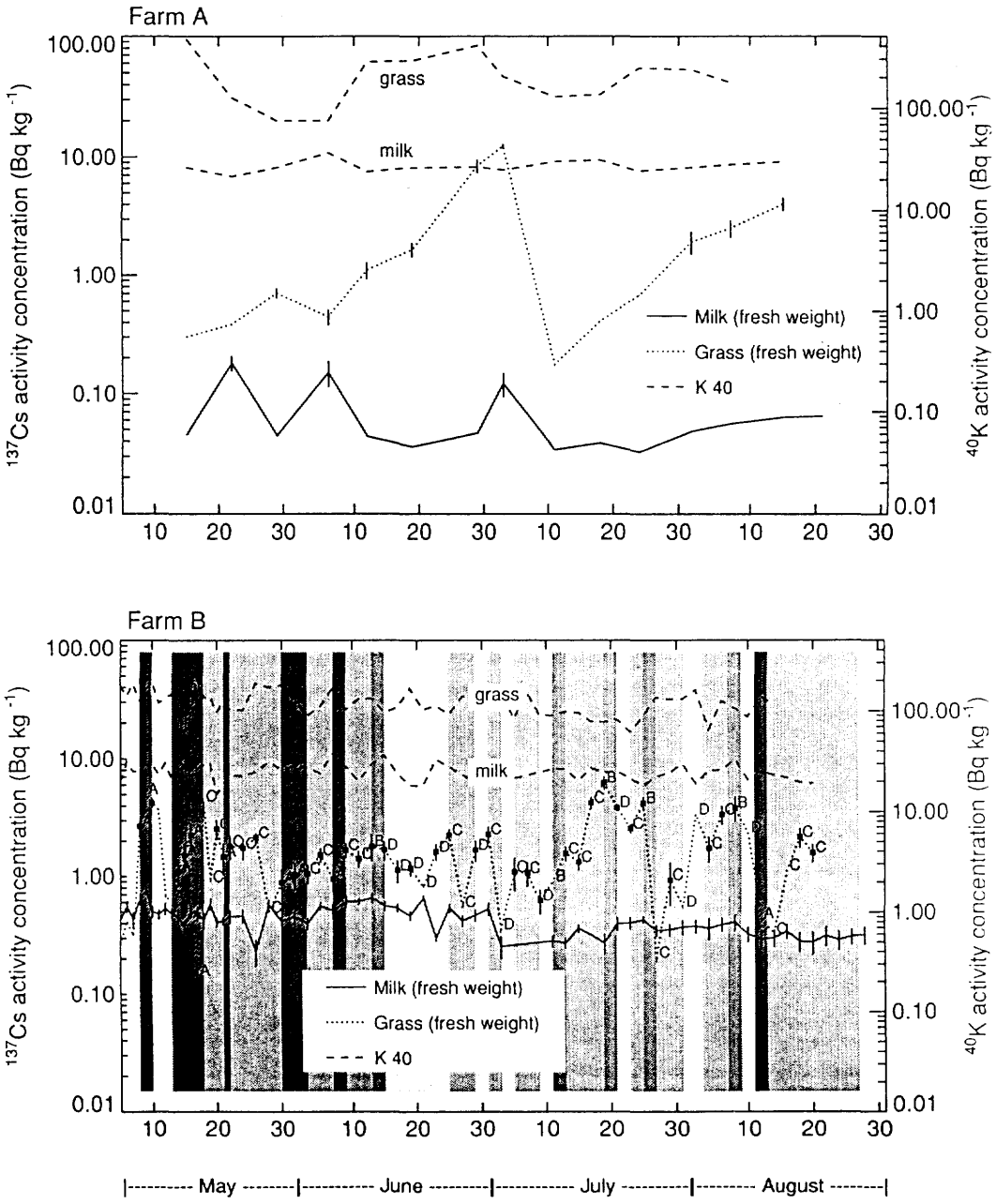
Samples will be kept for eventually soil adhesion measurements.

Publications

Hove, K., Strand, P., Voigt, G., Jones, B.-E.V., Howard, B.J., Segal, M.G., Pollaris, K. & Pearce, J. 1993. Countermeasures for reducing radioactive contamination of farm animal products. *Sci. Tot. Environ.*, **137.**

Voigt, G., Howard, B.J., Vandecasteele, C.M., Mayes, R.W., Belli, M., Sansone, U., Stakelum, G., Colgan, P.A., Assimakopoulos, N.M.J., Crout, B.E-V. Jones & Hove, K. in press. Factors affecting radiocaesium transfer to ruminants. In: Proc. Ann. 25th Meet. Fachverband fuer Strahlenschutz, 28-30 Sep 1993, Rügen, Germany.

Figure 1. Changes with time in the radiocaesium and ^{40}K activity concentrations in vegetation and milk collected from Farm A and Farm B.



Head of Project 6: C. Vandecasteele

II. Objectives of the reporting period

1. To compare the gastro-intestinal bio-availability of radiostrontium when it was (1) biologically incorporated into plant material (clover) and fed to sheep as hay, (2) when the clover was fed as silage, and (3) when the strontium was administered in the ionic form. Highly contaminated plants had to be produced under greenhouse conditions by growing red clover in nutrient solution.
2. The differences in gastro-intestinal availability of ionic strontium in sheep was compared to that of radiostrontium bioaccumulated into different forages species using the true absorption coefficient.

III. Objectives of the next reporting period :

To determine the kinetics of radioiodine in cow's milk throughout the lactation period, from calving till drying. Six pregnant cows will be contaminated at regular intervals with ^{131}I. The animals will be divided into two groups. The first group will receive fluctuating amounts of stable iodine, depending on the I content in the different constituents of the feed in regime and the variations of this regime during the milk production cycle. The second group will be given a constant I daily dose by supplementing the feed with KI. The secretion of ^{131}I will be followed in milk, faeces, urine and blood.

IV. Progress achieved including publications :

In order to prepare the experiment, red clover was sown and grown in an open field. At harvest the clover production was divided into two parts : the first one was taken from the field after one day of drying and poured into 1 m^3 plastic tanks for silaging and the second part was dried in the field as hay.

Meanwhile, highly contaminated material was obtained in the greenhouse by growing clover in 1 m^2 PVC trays (50 l) containing artificially contaminated nutrient solutions (740 kBq ^{85}Sr/l). Polyacrylamide gel was used as a suitable, but inert, foothold to the plants. The first cut was used for silage production following the same technique as for the uncontaminated material, but using a 10 l vessel for the fermentation of the plant material. The second harvest, three weeks later, was dried in an oven at 50°C.

Daily doses administered to the animals (containing about 20 kBq ^{85}Sr for clover hay and about 35 kBq ^{85}Sr for clover silage) were prepared in advance by counting each individual dose on a NaI detector and adjusting its quantity of plant material to the requested activity value (within a range of plus or minus 2%). Eight supplementary doses were prepared to allow the random sampling of doses for radioactivity measurements using a standard geometry.

Twelve female sheep of the same age were selected from the flock of the CEN/SCK and placed on metabolism cages in the experimental stable. The animals were divided into three equal groups of four animals :

- The first group received biologically incorporated strontium (approximately 35 kBq ^{85}Sr/d) in clover silage. The highly radioactive plant material (about 50 g wet weight) was given in the morning. The animals then received small amounts of beet pulp and uncontaminated clover silage *ad libitum.*

- The second group was administered biologically incorporated strontium (approximately 20 kBq ^{85}Sr/d) as clover hay. The contaminated plant material (about 10 g) was given in the morning. The animals then received small amounts of beet pulp and uncontaminated clover hay *ad libitum.*

- The third group was dosed every morning with ionic strontium as $^{85}SrCl_2$ (34 kBq/d) spiked on beet pulp. Two sheep from this last group were fed uncontaminated clover hay *ad libitum* while the other two were given uncontaminated clover silage.

All twelve animals were continuously perfused with NaCl isotonic solution (0.9% NaCl) containing $^{89}SrCl_2$ through a catheter inserted into the jugular vein The perfusion rate of about 200µl/min (300 ml/d) was controlled by multichannel peristaltic pumps and provided a daily injection of 100 kBq ^{89}Sr directly into the blood. The oral dosings with the different forms of ^{85}Sr and the intravenous perfusion with ^{89}Sr were started at the same time and lasted for 8 days. At the end of the last day, the animals were sacrificed.

Faeces and urine (bladder catheter), free of cross-contamination, were collected daily. After weighing, 1 l sub-samples of urine were acidified with 5 ml concentrated HNO_3 , to lower the pH to 6.5 and avoid losses of Sr, and stored at 4°C until strontium extraction. Fifteen ml urine sub-samples were taken at the same time and used for direct radioactivity determination by gamma spectrometry. One kg sub-samples of faeces were oven dried (105°C x 24 h) and ground. Blood and different organs (including muscles, bones, bone marrow, kidneys, liver, ...) were sampled at sacrifice. These samples were lyophilised and eventually ground.

Based on the gamma spectrometry measurements of the 15 ml urine samples, the ^{85}Sr and ^{89}Sr (through the interference in the compton region of the ^{85}Sr) were roughly estimated. These preliminary results show a comparable excretion of the intravenously administered ^{89}Sr in urine, for each sheep group and suggest a comparable metabolic response of the animals within the three groups. Related to the activity of the orally administered ^{85}Sr, the concentration measured in urine suggest a similar availability of strontium in clover silage and hay, as well as, from the ionic strontium.

These results are very preliminary and larger samples of urine and other material collected are presently processed radiochemically to concentrate and extract radiostrontium. These samples will be soon measured by gamma spectrometry (^{85}Sr) and liquid scintillation counting (^{89}Sr).

Publications

Vandecasteele C.M. (1992) Post-Chernobyl field and experimental studies at Belgium's CEN/SCK farm, Nuclear European Worldscan, n° 9-10: 90-91.

Burton O., Vandecasteele C.M., Van Hees M., Lambotte J.M. & Kirchmann R. (1992) Behaviour of radiocaesium and radiostrontium with aerosol particles deposited in a pasture ecosystem. In Proceedings of the Soviet Branch of the International Union of Radioecologists Seminar on : "Radioecology and Countermeasures". Kiev 27 April - 2 May 1991. IUR DOC p 103-115.

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Hove, K., Strand, P., Voigt, G., Jones, B.-E.V., Howard, B.J., Segal, M.G., Pollaris, K. & Pearce, J. 1993. Countermeasures for reducing radioactive contamination of farm animal products. *Sci. Tot. Environ.*, **137.**

Voigt, G., Howard, B.J., Vandecasteele, C.M., Mayes, R.W., Belli, M., Sansone, U., Stakelum, G., Colgan, P.A., Assimakopoulos, N.M.J., Crout, B.E-V. Jones & Hove, K. in press. Factors affecting radiocaesium transfer to ruminants. In: Proc. Ann. 25th Meet. Fachverband fuer Strahlenschutz, 28-30 Sep 1993, Rügen, Germany.

Head of Project 7: K. Hove

II Objectives for the reporting period

1. Conduct experiments designed to test the effect of ^{133}Cs dosing on the transfer of ^{134}Cs to meat and milk.
2. Review the litreature for posible Sr-binders. Test possible Sr-binders in vivo using the lactating goat setup previously used at our University for Cs-binders.

III Objectives for the next reporting period

1. Perform the remaining analysis and calculations of samples and results from the experiment testing the effect of ^{133}Cs dosing on the transfer to meat and milk.
2. Testing of the Sr-binding effects of synthetic zeolites (7 different types) and natural clinoptilolites (2) in lactating goats.
3. Test Pillared layered clays (PILC's) developed at NPL, Ioannina, as binders for Sr and Cs in collaboration with the group in Ioannina.

IV Progress achieved including publications.

Caesium metabolism study

The goats were given a diet containing 0.35 mg of ^{133}Cs and supplemented with 0-2000 mg/d of ^{133}Cs. In addition ^{134}Cs (14100 Bq/d) was fed daily with the concentrate for 50 d in order to obtain equilibrium between intake and ^{134}Cs levels in meat. The goats were divided into 7 groups of 4 animals, and kept in the barn for 42 d where milk, blood and live monitoring measurements were performed 3 times each week. After 42 d the animals were taken into metabolism cages for 7 d and given an single intravenous injection of ^{137}Cs for measurements of true absorption. During the period in metabolism cages samples of milk, urine, faeces and blood were taken. At the termination of the experiments one muscle biopsy was taken.

Data from the experiment are given in Table 1. The mean apparent absorption (A_a) of ^{134}Cs ranged from 0.65 to 0.75 and the mean true absorption based on milk measurements (A_t) ranged from 0.89 to 1.33. These values are somewhat higher than those reported in non-lactating sheep, and do agree with the concept of a nearly complete availability of ionised Cs in ruminants. Transfer coefficients to milk were within earlier observed ranges for ionic Cs even when 2000 mg/d of ^{133}Cs was given. The mean transfer coefficients to meat showed the same result.

Table 1. Mean absorption measurements and transfer coefficients to milk (F_f) and meat (F_m) from lactating goats given a dairy ration supplemented with different amounts of ^{133}Cs.

Group	^{133}Cs mg/d	A_a	A_t	F_m ^{133}Cs	F_m ^{134}Cs	F_f ^{134}Cs
1	0	0.73	0.99	-	0.08	0.35
2	5	0.74	0.97	0.17	0.09	0.31
3	20	0.74	1.00	0.17	0.09	0.25
4	50	0.72	1.06	0.10	0.09	0.32
5	100	0.71	0.94	0.11	0.11	0.35
6	500	0.75	1.33	0.12	0.09	0.36
7	2000	0.65	0.89	0.07	0.11	0.39

The highest levels of ^{133}Cs given in the present experiment were 40 times higher than in experiments where once weekly additions of ^{133}Cs have been reported to decrease F_f and increase F_m. The present study showed, however, that ^{133}Cs given daily on the feed did not affect the transfer of ^{134}Cs to meat and milk in dairy goats.

Testing of strontium binders

Possible Sr binders were tested in lactating goats fed ionic ^{85}Sr. The effect of the binders were measured as reduced transfer of ^{85}Sr to milk compared with those determined for control animals. The experiment included 26 goats in 13 groups and lasted for 21 d. The goats were fed individually. Milk samples were taken 3 times weekly. Six compounds were tested during the first reporting period.

Table 2. Effects of potential Sr-binders on ^{85}Sr transfer to milk given as a percentage of transfer measured in control goats

Substance	**Source**	**Amount g/d**	**Reduction**
Na-rhodizonic acid	Sigma	1.7	ns
K-rhodizonic acid	Sigma	1.7	ns
Sb_2O_5	Strelko	0.25	ns
	Kiev	1.0	ns
Humalite	Priester	1.0	ns
	Kiev	2.0	ns
		4.0	ns
Zeolite A (Na)	Degussa	2.0	30%
Zeolite Y (Na)	Degussa	2.0	30%

Only the zeolites reduced the transfer of ^{85}Sr to milk in the doses tested. Further experiments will be carried out with other natural and synthetic zeolites and with higher doses. The other compounds would not be feasible in higher doses either due to toxicity or to economic consideration.

Pubications

Voigt, G., Howard, B.J., Vandecasteele, C.M., Mayes, R.W., Belli, M., Sansone, U., Stakelum, G., Colgan, P.A., Assimakopoulos, N.M.J., Crout, B.E-V. Jones & Hove, K. in press. Factors affecting radiocaesium transfer to ruminants. In: Proc. Ann. 25th Meet. Fachverband fuer Strahlenschutz, 28-30 Sep 1993, Rügen, Germany.

Head of Project 8: T. Hinton

II. Objectives for this reporting period:

1) Conduct a literature review of Ti and Sc uptake by plants to evaluate their suitability as tracers of soil loading onto plants

2) Determine why some Ti estimates of soil loading, determined by the 'CEC Animal Group' during the last contract period, grossly over predicted the contribution of resuspension to the overall Cs contamination of the plant.

3) Develop a calibration method for a technique to determine the particle size of soil adhering to leaf surfaces.

III. Objectives for the next reporting period:

1) Finish refining the methods for determining the particle size distribution of soil adhering to plant surfaces

2) Utilise these techniques in manipulative experiments that examine the interaction of decreased Cs concentration in heavily grazed pastures, due to growth dilution of the vegetation, and increased Cs-contaminated soil adhering to the plants, due to increased animal-induced resuspension.

IV. Progress Made including publications:

Literature Review on the Applicability of Titanium, Scandium and Plutonium as Tracers of Soil Loading

These elements have very low biological availability and are not readily absorbed through leaf surfaces or taken up by plant roots. Therefore, any detection of these elements when the plant is analysed is considered to be a measure of the elements in soil adhering to the plant. The applicability of elements as tracers of soil contamination of herbage is limited to those with low plant/soil ratios; elements with ratios of 10^{-3} to 10^{-5} are likely candidates. Quality data correlating soil concentrations to plant concentrations are limited, however, because very few studies have separated surface contamination from endogenous contamination within the plant material. Table 1 presents Ti, Sc and Pu concentrations in soils and plants for those studies that reported both. The accuracy's of reported plant concentrations are questionable, however, because few researchers reported if plants were washed prior to analysis.

Titanium is the most widely used tracer of soil movement. Average concentrations in soils seem to be about 5000 ppm and endogenous plant concentrations about 10 ppm, resulting in plant/soil ratio of $2*10^{-3}$. This gives credence to the claim that Ti is only sparingly soluble in soils, considered relatively unavailable to plants, and not readily mobile in them. Ti is soluble at a pH < 1.5 or > 14, between these values hydrated oxides precipitate.

Scandium is much less abundant, yet its occurrence is strongly correlated to other elements associated with soil. The average Sc content in world soils from data reviewed by Horovitz et al. (1975) was 7 ppm with a range of 3 - 50 ppm. Early data indicate a mean of 0.008 ppm for terrestrial plants. Horovitz et al. (1975) found Sc concentrations in the leaves of 12 different plant species to be

0.039 ± 0.019, although the methodologies do not state if the plant material was washed prior to analysis. Where washing was part of the procedures, Sc concentrations in dried plants ranged from 0.003 to 0.14 ppm. The better data suggest a mean plant/soil ratio of 10^{-3}.

Plutonium is a product of nuclear technology and is not a normal component of the earth's crust. Soil concentrations are determined by nuclear weapons fallout or other anthropogenically enhanced sources. Numerous studies have examined the plant uptake of Pu and found Pu to be relatively immobile, with plant/soil ratios ranging from 10^{-3} to 10^{-6} (Table 1). The behavior of Pu is governed by processes that influence the chemistry of Pu^{4+}. Only when Pu^{4+} is stabilised by natural ligands such as humic and fulvic acids can it remain in the soluble state and be taken up by plant roots.. The amount of hydrolized Pu in the soil is extremely small; a typical soil analysis shows only 0.01% present in solution and much of this is in colloidal form.

Table 1. Plant to soil concentration ratios for Ti, Sc, and Pu

	SOIL (ppm)	PLANT (ppm)	PLANT/SOIL	REFERENCE
TITANIUM				
	10,000	1	$1 * 10^{-4}$	Mitchell 1960
	6,000	20 -190	$2 * 10^{-2}$	Dumon & Ernest 1988
	5,000	1	$2 * 10^{-4}$	Bowen 1966
	4785	218	$5 * 10^{-2}$	Mayland & Sneva 1983
	4525 ± 1763	780 ± 710	$1 * 10^{-1}$	Tonkonozhenko 1974
	2880	9 **	$3 * 10^{-3}$	Arthur & Alldredge 1979
	2477	125	$5 * 10^{-2}$	Cary et al. 1986
SCANDIUM				
	7	0.008	$1 * 10^{-3}$	Bowen 1966
	2.1 - 26.2	0.04 - 2.5	$10^{-3} - 10^{-1}$	Horovitz 1975
	10 -15	10	1	Shacklette 1966
	0.1 - 200	0.01 - 1	$10^{-3} - 10^{-1}$	Markert 1992
PLUTONIUM				
	-	-	$10^{-4} - 10^{-3}$	Rediske et al. 1955
	-	-	$10^{-5} - 10^{-2}$	Fedorov et al. 1986
	-	-	10^{-4}	Vyas and Mistry 1978
	-	-	$10^{-4} - 10^{-3}$	Okajima et al. 1989
	-	-	$10^{-5} - 10^{-4}$	Brown & McFarlane 1977
	-	-	$10^{-8} - 10^{-6}$	Schulz, et al 1975
	-	-	$10^{-9} - 10^{-6}$	Cline & Schreckhise 1987

** plants were ultrasonically washed

Analysis of samples from Ireland and England

Specific Ireland and UK soils from which previous mass loading estimates were unreasonably high have been separated by particle size using mechanical sieves and a gravitational settling method. Analysis for ^{137}Cs revealed an unusual uniform distribution. It is probable that the Ti tracer is distributed differently and thus caused the unreasonable soil mass loading estimates. Ti

analysis of the samples will be possible after the first period funding is received. Our hypothesis concerning the problem stems from the following experiment:

Experimental Comparison of Soil Mass Loading Techniques

We compared four common techniques used to estimate soil mass loadings onto vegetation and found significant differences in their estimates. Samples were collected from a pasture near Chernobyl and mass loadings were estimated from Pu content determined by alpha spectroscopy, Ti analysis via ICP, neutron activation analysis for Sc, and a simple washing method. The washing technique resulted in a mass loading 30 times greater than that estimated from using Pu, and 10 times greater than when Ti was used. Analysis of variance of the log-transformed data revealed that differences among the techniques were significant at $p < 0.001$. Bonferroni tests were employed to examine individual differences. Significant differences occurred between each technique with the exception of Sc and Ti ($p = 0.055$).

Soil cores taken at the time of plant sampling were frozen and the upper 0.5 cm of soil removed for analysis. Soil samples were separated by particle size using a mechanical vibrator and sieves of 2.00, 0.500, 0.250, 0.125, and 0.056 mm in diameter. Subsequent analysis of the radionuclide concentrations in the soil revealed a strong dependence upon particle size. Table 2 shows that for all isotopes examined, including the stable tracers Ti and Sc, concentrations were greatest at the smallest soil particle sizes. This particle size dependency of concentration affects the estimation of soil mass loading onto plants. A factor of 10 difference in mass loadings estimates is possible for the same samples, depending on what size soil fraction is used in the soil mass loading calculation.

Table 2. Concentration of elements by soil particle size (mean ± SE).

	Soil Particle Size (mm)			
Element	< 0.056	0.056 - 0.125	0.125 - 0.250	> 0.250
Ti(mg/g)	2220 ± 37	884 ± 18367 ± 90	135 ± 10	
Sc(ug/g)	3.2 ± 0.1	1.4 ± 0.30.6 ± 0.2	0.4 ± 0.2	
^{238}Pu(Bq/g)	0.55 ± 0.23	0.13 ± 0.08	0.023 ± 0.004	0.020± 0.007
^{239}Pu(Bq/g)	1.15 ± 0.48	0.27 ± 0.16	0.045 ± 0.011	0.034 ± 0.012
^{134}Cs(Bq/g)	32.8 ± 5.0	13.4 ± 3.3	3.3 ± 0.5	nc(1)
^{137}Cs(Bq/g)	517 ± 81	188 ± 40	52 ± 7	nc
^{241}Am(Bq/g)	19 ± 4	4 ± 2	0.3 ± 0.1	nc
^{106}Ru(Bq/g)	28 ± 5	8 ± 3	0.5 ± 0.2	nc

(1) not calculated

It is important to determine if the soil tracers used (Sc, Ti, and Pu) were distributed in the soil similarly to the radionuclides of interest. Table 3 presents

Pearson correlation coefficients for all of the radionuclides examined and the stable tracers Sc and Ti. If Cs were the radionuclide of interest, Table 3 shows that the distribution of ^{239}Pu in the soil correlated more closely to that of Cs (r^2 = 0.92) than did Ti (r^2 = 0.58) or Sc (r^2 = 0.67). Not accounting for the correlation between the soil distribution of the tracer and the radionuclide of interest might explain why some soils from Ireland and England presented unreasonable mass loading amounts.

Table 3. Pearson correlation coefficients are presented for the distribution of elements by soil particle sizes. When comparing any two elements, the closer the value to 1.0 the stronger the correlation.

	^{241}Am	^{134}Cs	^{137}Cs	^{238}Pu	^{239}Pu	^{106}Ru	Sc	Ti
^{241}Am	1.00							
^{134}Cs	0.56	1.00						
^{137}Cs	0.56	1.00	1.00					
^{238}Pu	0.61	0.90	0.90	1.00				
^{239}Pu	0.61	0.92	0.92	0.98	1.00			
^{106}Ru	0.71	0.77	0.77	0.82	0.82	1.00		
Sc	0.30	0.67	0.67	0.65	0.68	0.50	1.00	
Ti	0.21	0.58	0.58	0.55	0.62	0.42	0.95	1.00

Calibration of Technique to Determine the Particle Size of Soil Adhering to Plants

We have been exploring a technique to determine the particle size of soil adhering to plant surfaces. Such information would allow us to considerably increase the accuracy of our soil mass loading estimates. Our technique employs a liquid plastic applied to the leaf surfaces. When the plastic dries and is removed, all the surficial particles that were attached to the leaf are embedded within the plastic. Scanning electron microscopy of the plastic is then used to examine the particle sizes. The technique needed calibrating in that we were unable to interpret the SEM photos. Attempts at calibration were not successful, and we have now altered the technique so that the plastic is chemically treated to remove organic material captured on the leaf. The remaining mineral material is quantified using a computer-coupled light microscope. The technique is still being refined.

Publications

Hinton, T. G., P. Kopp, S. Ibrahim, I. Bubryak, A. Syomov, and L. Tobler. 1993. Contaminated soil on Chernobyl Vegetation. Proceedings of the 26th Topical Meeting of the Health Physics Society. Jan 24-28, 1993, Coeur d'Alene, Idaho, USA. pp407-421.

Progress Report

Contract: FI3P-CT920016 Sector: A25

Title: Deposition of radionuclides on tree canopies and their subsequent fate in forest ecosystems - Further studies.

1)	Minski	IMPCOL
2)	Rauret	Univ. Barcelona
3)	Ronneau	Univ. Louvain (UCL) - LLN

I. Summary of Project Global Objectives and Achievements

During the 1990-92 period this group addressed the capture of radioactive aerosols by forest canopies and their subsequent fate. Due to lack of information on these topics following the Chernobyl accident this study was of major importance, as confirmed by data recently available from the USSR. The 1990-92 contract focused on the ability of individual trees and 'model' forest canopies to intercept aerosols depositing from the atmosphere under dry conditions and to subsequent retention of these deposits over time. In addition, the Louvain-la-Neuve and Barcelona groups extended their interests to an examination of the physical migration of caesium in multi-layer forest soils typical of north and south Europe. As a result of these findings the objectives of the 1992-94 contract are to extend this work to both near and far field situations. The complexity of the dry deposition results for the earlier period was greater than initially expected and in this work the need to elucidate further the effects of canopy morphology and related micro meteorological conditions will be addressed, with particular reference to identifying small scale diffusion and impaction of aerosols **within** canopies. Further wind tunnel work at LLN and IC-CARE using thermogeneration of UO_2 aerosols, together with delayed neutron counting techniques for ultimate sensitivity will enable more detailed studies of dry deposition. In addition the use of gusting facilities at IC-CARE will permit studies to be made under 'controlled' conditions more realistic of true field conditions. CEA will extend wind tunnel findings to real canopies and the data used in the validation of an atmospheric deposition model developed at Fontenay-aux-Roses. Radiocontamination of forest soils in the new field situation will be addressed and vitrified hot particles developed using the thermogeneration technique at LCN, will be used in studies of fuel fragment behaviour in forest soils.

Head of project 1: Miss. Minski

II. Objectives for the reporting period

The main objective has been the development of an aerosol generation technique for the production of a monodisperse uranium aerosol in sufficient quantity to allow sensitive detection once liberated in the wind tunnel. The simultaneous development of analytical protocols, based on the technique of delayed neutron counting, has also been a priority and the desired endpoint of the current reporting period was the ability to carry out full scale wind tunnel deposition studies using the newly developed uranium aerosol by the end of the first year of the current contract. As a test of the readiness and compatibility of the new aerosol generation and detection techniques it was intended to carry out a trial deposition experiment using a canopy comprising Norway spruce saplings, as used in the previous contractual period.

Objectives for the next period are the performance of a full scale dry deposition experiment using Norway spruce saplings in which aerosol generation and detection will have been 'fine-tuned' to give the lowest detection limits achievable. This will allow high resolution sampling of the tree tissues to test the model of canopy deposition developed by Dr. Belot (CEA, Fontenay-aux-Roses, France) during the last contractual period. In addition the effects of forest edges on dry deposition will be examined as well as detailed studies of resuspension at different levels within the canopy. All of these studies will make use of the uranium aerosol generation technique developed during the current reporting period.

III. Progress achieved including publications

In order to ensure continuity with dry deposition studies in the previous contractual period the production of uranium aerosols with an aerodynamic diameter of 1 μm was necessary. For dry deposition and prompt resuspension studies the exact chemical form of this aerosol is largely unimportant as the detection method of delayed neutron counting merely requires that ^{235}U is present as a tracer in the aerosol. A simple and convenient aerosol generator was designed using a commercially available sidestream nebulizer (Medic-Aid, West Sussex, UK) to produce dry aerosols from various aqueous solutions.

Experimental apparatus

The aerosol generation system developed over the last 10 - 12 months consists of a) a sidestream nebulizer connected to a clean air supply, b) a tube where the dispersion and transport of the primarily produced aerosol takes place by mixing clean air immediately after the nebulization of the aqueous solution, c) a furnace to completely dry the generated particles, d) a ^{85}K source to reduce any static charge on the particles and e) a cylinder for collection and sampling of the final aerosol. The assembly of the aerosol generation equipment thus achieved is shown in Figure 1.

This system was tested with lithium carbonate and uranyl acetate solutions to produce particles in the size range of 0.13 - 1.37 μm volume median diameter with a geometric standard deviation (GSD) of 1.4 - 2.0. The solutions were prepared by dissolving a known amount (10, 100, 300, 500 and 800 mg) of the material in 100 ml de-ionised water. The resulting concentrations correspond to 0.1, 1, 3, 5 and 8 gl^{-1}. For all experiments the reservoir of the nebulizer was initially filled with 5 ml solution. The nebulizer produced an aerosol flow rate of 3 1 min^{-1}, which was immediately diluted with clean air. The volume median diameter (VMD) and the mass median aerodynamic diameter (MMAD) of the aerosols so produced were measured using a Laser Aerosol Spectrometer (LAS -X) and an Aerodynamic Particle Sizer (APS, model 3302), respectively. Aerosol samples were also taken on 47 mm diameter, 0.2 μm porosity Nucleopore filters for subsequent examination by scanning electron microscopy (SEM). This gave details about the shape and structure of the aerosols produced.

Results and discussion

In our experiments we aimed to evaluate the effect of concentration, temperature and air dilution rates on the aerosol particle size. Numerous experiments were conducted to generate aerosols from lithium carbonate and uranyl acetate solutions at ambient laboratory temperature and at furnace temperatures of 200 and 400^{0}C. Diluting air was added at flow rates of 3, 15 and 25 1 min^{-1}.

i) Aerosol characteristics

The shape of the particles produced from the generator was investigated by SEM. Figure 2a is an SEM image of uranyl acetate aerosol particles, showing spherical shape and smooth surface. Figure 2b shows, for comparison, lithium carbonate particles, most of which were hollow spheres with a smooth exterior (Fig. 2b). Both uranyl acetate and lithium carbonate aerosols generated in this study showed a range of sizes although the particle size spectra were acceptably monodisperse. As shown in Figure 3, both VMD and MMAD increased as the concentrations of lithium carbonate and uranyl acetate are increased. A good correlation is also established between VMD and MMAD (Figure 4). The temperature (up to 400^{0}C) has no significant effect on either the shape or the size of the particles ($p<0.01$). Particle size is significantly affected ($p<0.01$) by the magnitude of the diluting airstream, both VMD and MMAD decreasing as the air flow rate increases (Figure 4). However, the effect is more pronounced between 3 and 15 or 25 1 min^{-1} than between 15 and 25 1 min^{-1}.

ii) Stability of the aerosol generator

In aerosol production, it is important that the particle size distribution of the final aerosol remains uniform as measured over a given period of unchanging operation of the generator. In our experiments we carried out tests on the stability of operation of the generator over a period of 48 hours during which aerodynamic particle diameter was monitored. These tests were undertaken with 0.1 g/l solution of uranyl acetate. The MMAD was measured every 30 minutes for the first 8 hours and every hour from then on. Typical size distributions for the number and the mass concentrations of the produced aerosol are shown in Figure 5. As shown in figure 6 the values of MMAD remained nearly constant even after refilling the reservoir of the nebulizer or switching off the generator for several hours.

iii) Aerosol deposition on spruce canopy - full-scale, trial experiment

A wind tunnel experiment has recently been carried out to investigate dry deposition processes of ^{235}U aerosol on a small Norway spruce (*Picea abies*) canopy. Saplings between 45 and 55 cm high were replaced in the wind tunnel and exposed to the aerosol generated from 0.1 g l^{-1} uranyl acetate solution using a single nebuliser. The latter produced uranium particles with MMAD of 1.18 ± 0.04 μm (mean of 267 measured values). The aerosol was liberated into the wind tunnel which was run at a constant wind speed of 5 ms^{-1} for a period

of 66 hours. Such a period of exposure was necessary to achieve a detectable uranyl acetate deposit as it was found that the air concentration produced by the single nebuliser was very low; in future a manifold will be constructed to allow the simultaneous operation of multiple nebulisers. 27 trees from the working section of the wind tunnel were sampled, with each individual tree being divided into five vertical layers, and allowed to air dry.

Results from this experiment are not yet available; deposition velocities (V_g) and interception fractions (f) will be calculated for total canopy and for 17 sub-components of the 5 vertical layers in each tree. V_g will also be determined for the ground surface between and beneath the trees by means of 7 cm diameter filter papers which were placed randomly prior to the experiment.

Summary

An aerosol generation system has been successfully developed to generate dry aerosols from aqueous solutions of uranyl acetate and lithium carbonate. The medical nebulizers used in this system produce droplets of many sizes and therefore, after evaporation, the resultant aerosol particles display a range of sizes; particle size spectra are, however, acceptably monodisperse. Both VMD and MMAD of the produced aerosols were a function of the concentration of the element in solution (NB the degree of solubility of the salt used is a limiting factor for the size of the particles to be produced). Particle diameters were also affected by the diluting air flow rate but not by temperature. Adequate drying usually required mixing the primary aerosol with clean dry air although 'warming' of an aerosol to accelerate the drying process, by passing it through a heated tube, may be necessary in some experiments. This could be the case when using a large number of nebulizers. However, further tests with higher temperatures (above 400^0C) should be carried out to investigate the effect on particle size and composition. The system permits the generation of aerosols over a long period of time with very low variation in the particle size. The time of exposure of trees to the aerosol inside the wind tunnel could be reduced by using solutions of higher concentrations with higher airflow rates and by using several nebulizers.

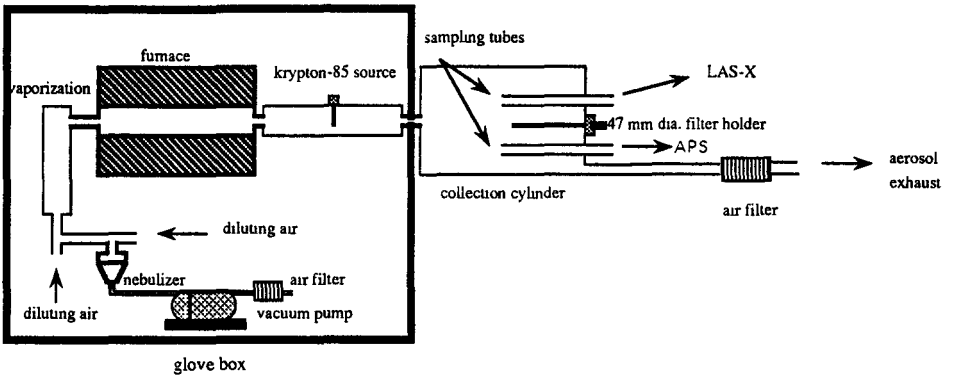

Figure 1. Schematic assembly of the aerosol generator.

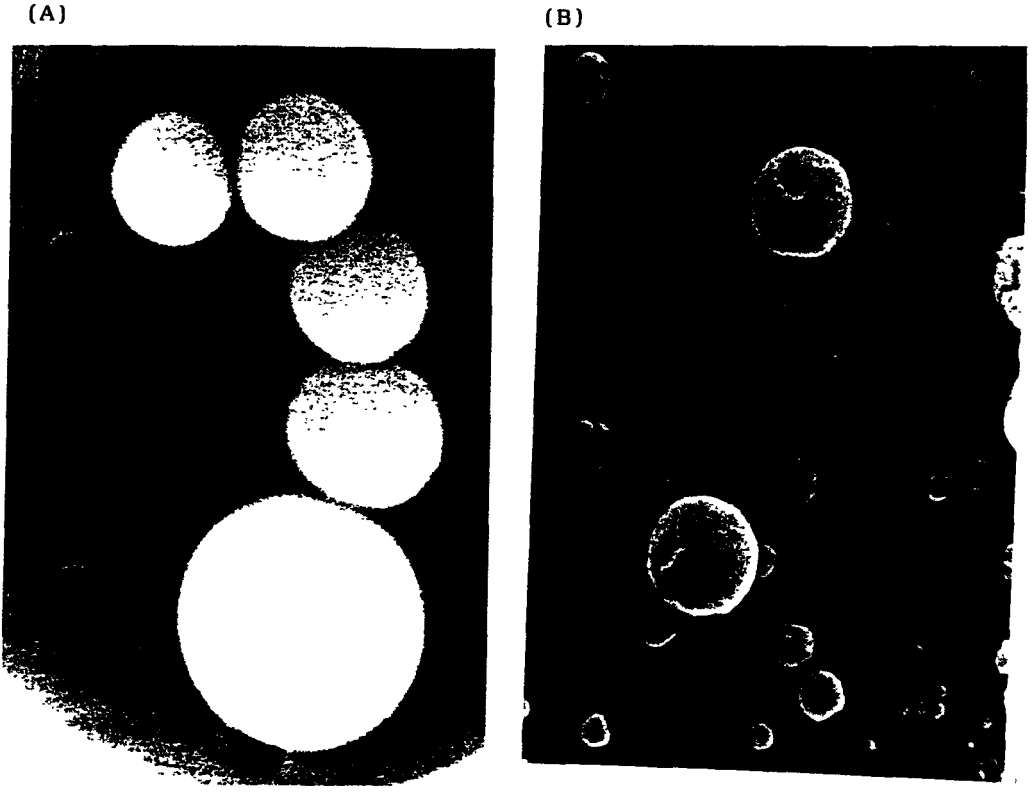

Figure 2. Scanning electron photographs for particles of a) uranyl acetate, 0.72 um VMD and b) lithium carbonate, 0.39 um VMD.

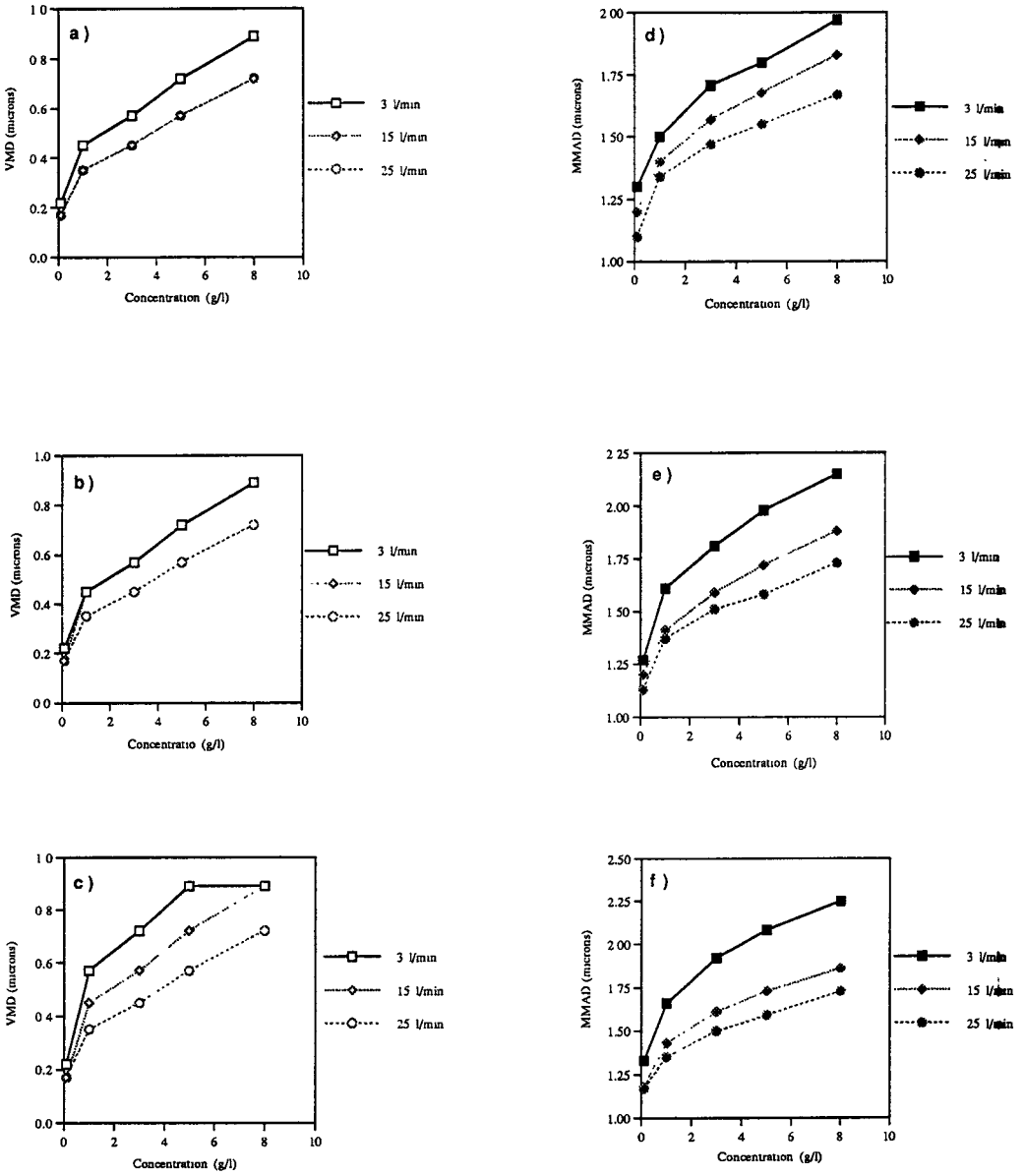

Figure 3. Relationship between VMD, MMAD and concentration for uranyl acetate aerosols (a) no heating b) at 200 C c) at 400 C for VMD and d) no heating e) at 200 C f) at 400 C)

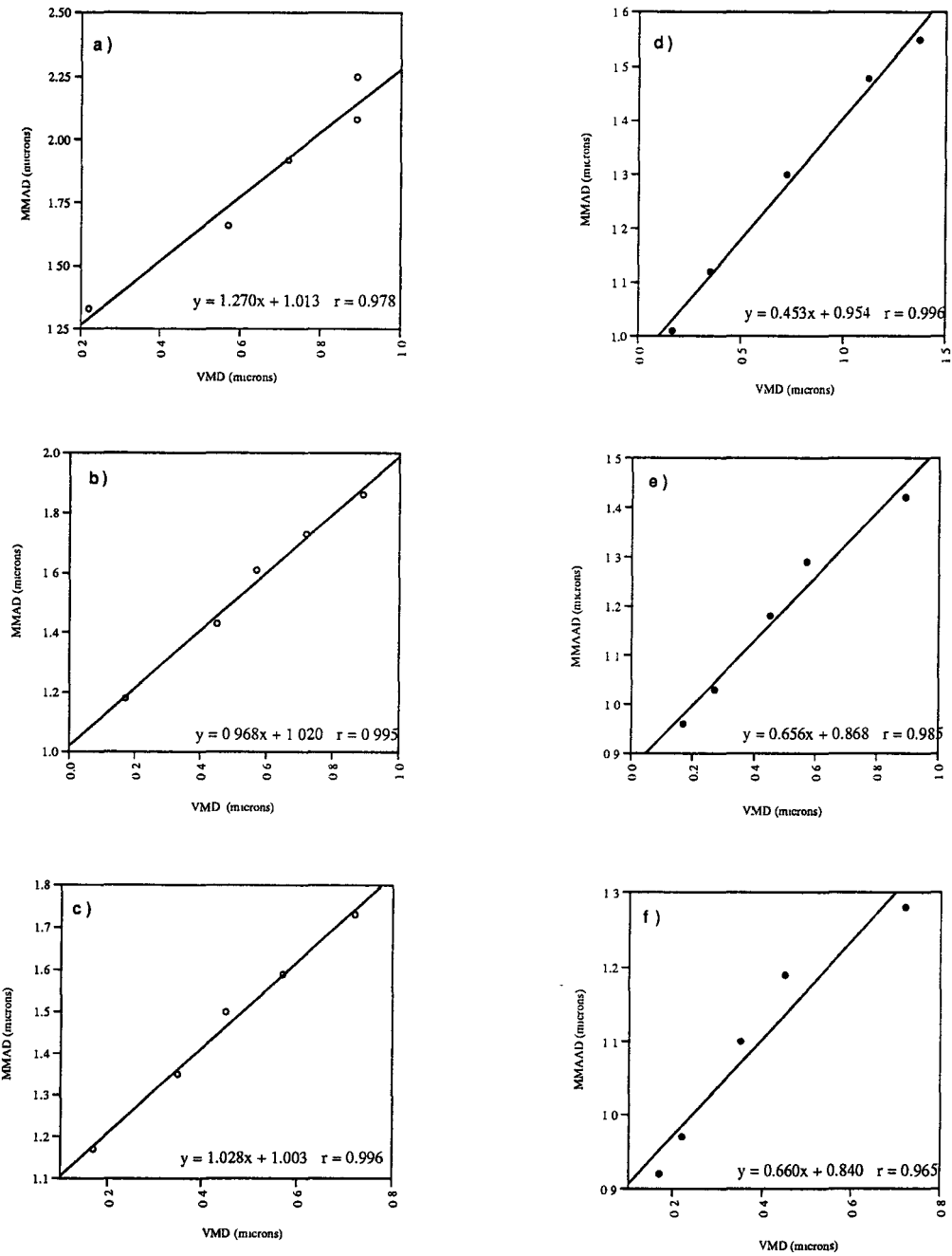

Figure 4. Relationship between MMAD and VMD of aerosols generated at 400°C with dispersion air of a) 3L/min b) 15L/min c) 25L/min for uranyl acetate and d) 3L/min e) 15L/min f) 25L/min for lithium carbonate.

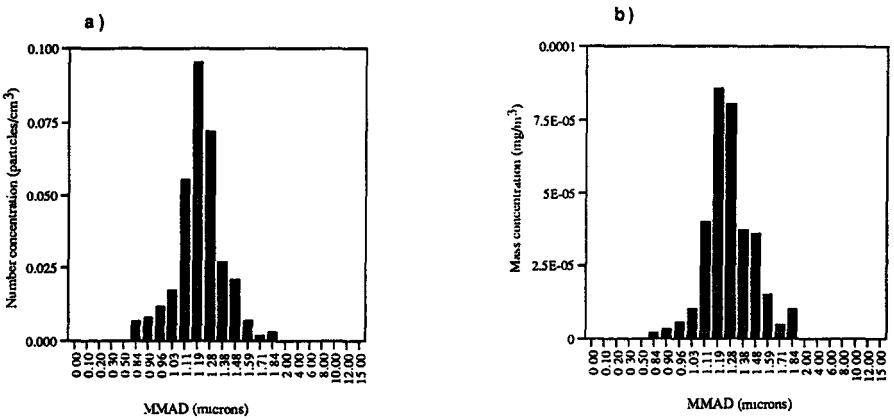

Figure 5. Particle size distribution of uranyl acetate aerosol (MMAD= 1.18 +_ 0.04) generated with no heating and with 15 L/min dispersion air. a) displays the number of particles per cm^3 per diameter channel and b) the mass of particles in mg/m^3

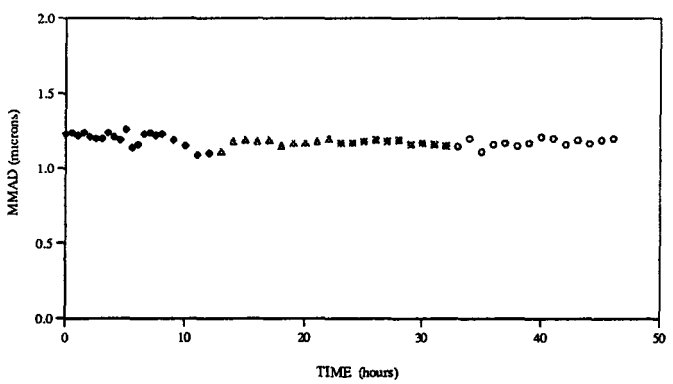

Figure 6. Variability of MMAD of uranyl acetate particles measured over a period of 48 hours (mean = 1.18 +- 0.04 microns). Difference in symbols indicate different times of sampling.

Head of the project 2: Dra. Rauret

II. Objectives for the reporting period.

1st. Study of ^{134}Cs and ^{110m}Ag migration in Mediterranean forest soils according to organic matter decomposition in the soil: a) completion of field sampling period, b) evaluation of methodology, c) study of litter decomposition processes and d) radionuclide migration in forest soil.

2nd. Measurement of soil water potential and temperature, in addition to a conventional meteorological parameters.

III. Objectives for the next period.

1st. End the study of radionuclide migration in Mediterranean forest soil.

2nd. Preliminary study of the behaviour of "hot particles" synthetized in Louvain-la-Neuve (Belgium) on holm oak leaves.

3rd. To determine the location of radionuclides in the decomposing leaves and F layer by solvent extraction in order to understand the mechanisms involved in their release from contaminated forest floor.

4rd. Modelling litter decomposition and radionuclides release in mediterranean conditions. 'SOIL' and 'DECO' models will be used for this purpose.

IV. Progress achieved including publications.

1st. Results of radionuclides migration and litter decomposition are presented, from the analysis of field incubation of artificially contaminated litter in open cylinders (with faunal activity), resin cylinders (no macrofauna) and standard litter bags.
^{134}Cs and ^{110m}Ag activity percentages of different forest soil layers at 330 and 450 days of incubation has been done.

a) Evaluation of the methodology.

Owing to the fact that to distinghish the original contaminated litter from the underlying original F layer in the open cylinders begin to be difficult along time, an increase of dispersion between replicates has been observed. During the previous period analyzed, the efectiveness of the resin bag to prevent de pass of burrowing macrofauna has been demonstrated. Nevertheless, in two last saplings, resin no avoid completly the pass of radiactivity in solution to the underlying forest floor, probably due to loss of homogeneity in the resin layer after some dried periods. This fact is more significant for ^{110m}Ag (fig. 1). due to the lower resin selectivity for this cation.

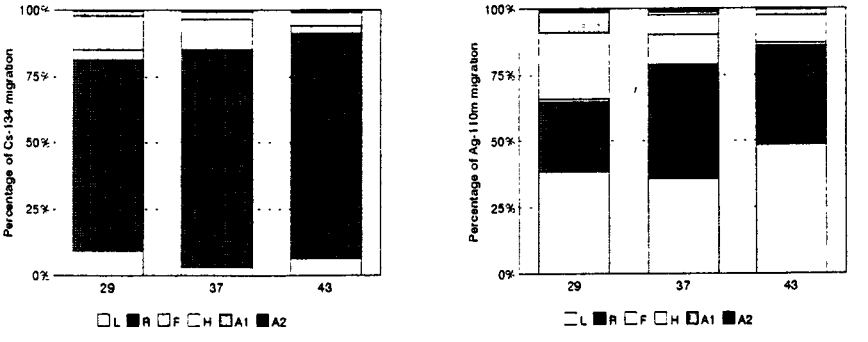

Fig.1. Percentages of radionuclides distribution at 450 days of incubation in cylinders with resin bags (29, 37 and 43 represents three replicates).

c) Litter decomposition.

During the first 232 days of incubation no differences in mass loss rates were observed between open and resin cilindre, nor with standard litter bags. However in spring, after litterfall, significant higher mass loss was observed in the open cilinder respect to resin cilinder and litter bags. In this period no litter decomposition were detected for resin cylinders and litter bags (fig. 2).
Macrofaunal faecal pellets are abundant in the remaining litter from the open cylinder, around 50 $mg*g^{-1}$ last sampling; in this way, litter comminution by soil fauna may increase litter decomposition in this type of cylinders.

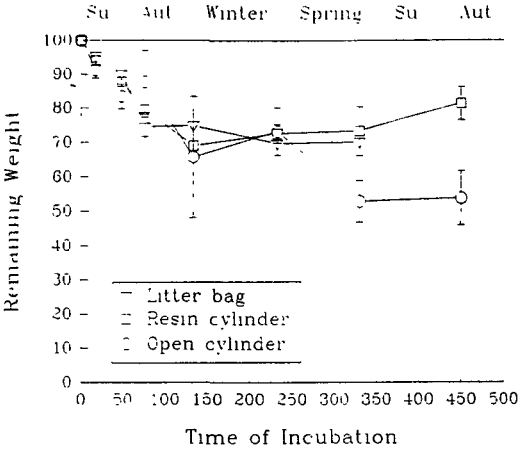

Fig. 2. Green leaves weight loss for the three methods used: litter bags, open cylinders and cylinders with exchange resin bags under the litter layer. Mean values with standard errors.

d) Radionuclides migration in the forest soil.

^{134}Cs migration showed no significant differences between the two types of incubation cylinders, indicating that Cs migration was predominantly in solution during the period studied (fig. 3a).
After 450 days of incubation only 15% of the initial radiocesium activity remains in the L layer. For the F layer, the activity accumulated remains around 40% but an important transfer of radiocesium from F to H layer was observed. Only 15 % arrived to the mineral soil (fig. 3b).

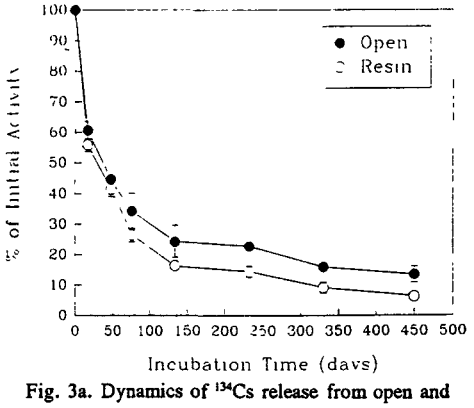

Fig. 3a. Dynamics of ^{134}Cs release from open and resin cylinders. Mean values with standard errors.

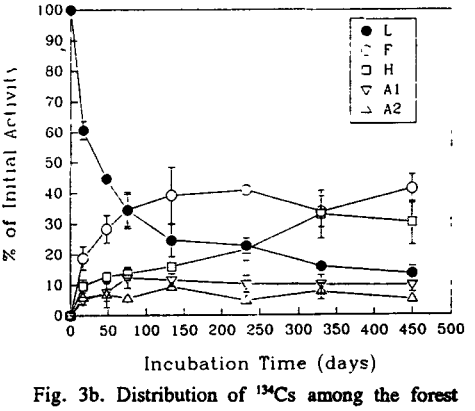

Fig. 3b. Distribution of ^{134}Cs among the forest floor and top soil layers during the incubation period. Samples from the open cylinders. Mean values with standard errors.

^{110m}Ag showed significant different migration pattern between open and resin cylinder after 232 days of incubation, but at the last sampling the percentage of ^{110m}Ag that remained in the L layer is similar for the two types of incubation (fig 4a).

^{110m}Ag migration in the soil profile (fig. 4b) was more gradual than ^{134}Cs and very little ^{110m}Ag contamination reached to the mineral soil. In two last samplings an important transfer of ^{110}Ag,like ^{134}Cs, from F to H layer was detected. The fact that the F layer accumulate the greater amount of radionuclides migrated from the L layer and because it is a substrat easy decomposable may play an important role in radionuclides retention/release dynamics.

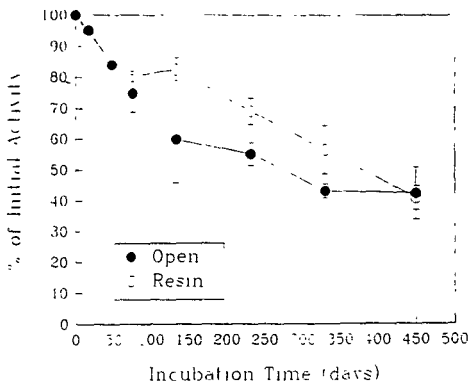

Fig. 4a. Dynamics of ^{110m}Ag release from open and resin cylinders. Mean values with standard errors.

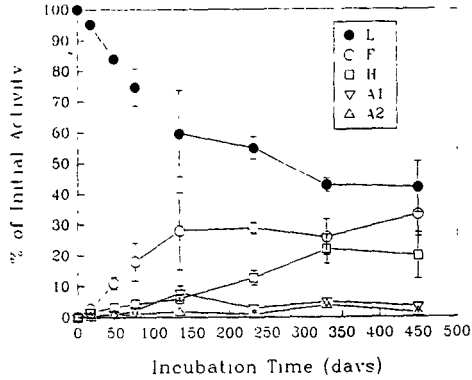

Fig. 4b. Distribution of ^{110m}Ag among the different layers during the incubation period. Samples from open cylinders. Mean values with standard errors.

Figure 5 shows the relationship between remaining activity and remaining weight for open and resin cylinder; significant correlation for the open cylinder was found but no for resin cylinder. In this cilinder radionuclides migration is no related to litter decomposition. It appears that in spite of possible particulate migration due to faunal activity and the role of soil fauna in litter decomposition in the open cylinder, migration in solution is the predominat process for both cylinders.

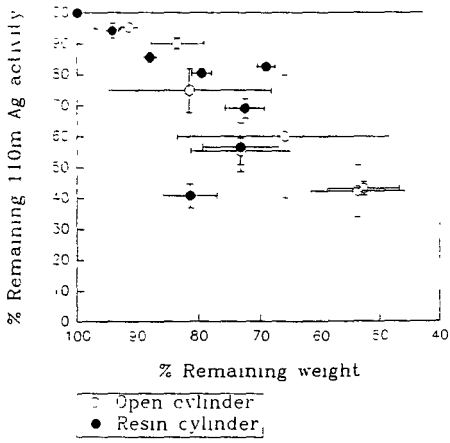

Fig. 5. Correlation between remaining weight and ^{110m}Ag activity in L layer along of incubation period. Mean values with standard errors.

2nd. Installation of psychrometers for soil water potential and temperature measurements has been done in the experimental forest site. During one year data will be collected two times every day for some periods coinciding wtih different seasons.

Head of Project 3: Professor C. Ronneau

II. Objectives for the reporting period

Globally, this study is aimed at defining the mechanisms of interception, transfer and recycling of radionuclides in forest ecosystems with particular emphasis on the fate of insoluble, vitrified UO_2 particles (containing fission products and trans-uranium elements) deposited onto forest soils.

The study is organised in three parts: i) defining the emission rates of radionuclides by neutron-irradiated UO_2 samples exposed to temperatures of 2000 - 2500°C (which approximate to those encountered during the Chernobyl accident), ii) determining the deposition rates onto foliar surfaces of both inactive aerosols (to evaluate micro-scale transfer mechanisms) and of aerosols and vapours emitted from activated UO_2 samples (to examine deposition of different fission products) and iii) defining the long-term behaviour in soils of fission products entrapped within vitrified 'hot particles' (either collected in the vicinity of the damaged reactor or synthesised within the laboratory from UO_2 tagged with ^{160}Tb).

III. Progress achieved including publications

I. Emission of fission products by irradiated UO_2

In a series of experiments small neutron-irradiated UO_2 samples were introduced into the graphite oven of an atomic absorption spectrophotometer and brought to temperatures of the order of 2000 - 2300°C under a flux of argon. The radioactive aerosols and vapours were subsequently submitted to a 'maturation' stage by contact with air into a second oven at about 700°C, in order to simulate the conditions likely to be met in the atmospheric plume emerging from a damaged reactor. The aerosols were filtered and the gases and vapours were absorbed by activated charcoal; the activity thus collected was determined by γ-ray spectrometry and the emission yields were calculated from these counts.

In parallel with these determinations the behaviour of radio-ruthenium was more closely examined as it appeared that, during the accident at Chernobyl, its behaviour was particularly influenced by the redox conditions in the atmosphere around the irradiated fuel. These experiments were performed in collaboration with the Khlopin Radium Institute of Saint-Petersburg, Russia. The results pertaining to this aspect of the study are presented in Annex I which has also been submitted for publication in the Journal of Environmental Radioactivity.

II. Deposition onto tree canopies

a) Deposition of inactive aerosols: As already described in the final report of the previous contract, lower wind speeds appear to enhance the deposition of small aerosols. Recent experiments have shown that this effect is still more obvious when considering different parts of twigs. In the case of spruce twigs the part of the needles close to the twig are more contaminated by aerosol deposition than the external parts. This may tentatively be explained by the slowing down of wind speed in the internal structure of the twig giving rise to an increased deposition by diffusion. Indeed, we used aerosols of the order of 0.2µm equivalent aerodynamic diameter (EAD) which is a size which is subject to diffusive transport. First results from this study are presented in Annex II.

b) Deposition of thermo-generated fission products from UO_2 samples: Experiments have been undertaken in order to study the deposition of fission products emitted by neutron-irradiated samples of ^{235}U-enriched UO_2. The vapours and aerosols emitted by

these samples are carried away, first by a flux of argon and then by air. They are then deposited onto spruce twigs under different wind velocities. γ-ray spectrometry allows the determination of deposition yields of the different radionuclides as a function of wind speed, moisture content of the air etc. Preliminary results are still too scarce to allow a coherent presentation here although full details will be given in the final report.

III. Lixiviation rates of 'hot' (fuel) particles

A few particles from the Chernobyl 30km zone have been submitted to different lixiviation tests. The results are still too fragmentary to give a coherent view of the situation. On the other hand, experiments have been conducted with hot particles synthesised in the laboratory which seem to give conclusive results about the poor leachability of vitrified UO_2 matrices. The results are presented in Annex III.
Another series of experiments has examined the action of microorganisms on these particles. These have demonstrated that, when placed in favourable conditions (ie. with a good nutrient supply), microbes are able to enhance significantly the lixiviation rates of fission products and trans-uranium elements from fuel particles. These experiments have been conducted in collaboration with the KRI of Saint-Petersburg and results will be presented in full in the final report.

ANNEX I
Oxidation-enhanced emission of ruthenium from nuclear fuel.

INTRODUCTION

The accident at the Chernobyl reactor ended in the release of about 2.8 10^{18} Bq of radioactivity in a complex, protracted process which lasted for a nine-day period. During the event, the temperature of the fuel, the volatilty of the different radionuclides and the blanket of materials spread in the crater were the main parameters to determine the emission rates. While about 3 % only of refractory elements were ejected from the reactor, the total inventory of rare gases was considered to have been released, together with 20 % of iodine, and 10 to 13 % of cesium (IAEA, 1986). Such estimations are of prime importance in assessing the consequences of accidents but they are reliable only if they are based on realistic assumptions about the chemical nature of the radionuclides.

Ruthenium is a particularly critical radionuclide, first because of its high versatility in changing oxidation states (up to VIII as in RuO_4) and in entering many stable complexes and, second, because of its easy tranfer into various ecosystems (see for example Coughtrey and Thorne, 1983; Fraizier, 1974). Another particularity is its high refractory character as an element (m.p.: 2310°C), in contrast with its tetroxide form (m.p.: 26°C).

As a refractory element, radio-ruthenium was estimated to have been released by the nuclear core at about the same rates as, for example, Zr, Ce, U and other trans-U elements. Ru emissions were considered to have practically stopped nine days after the initial burst of the reactor, together with the other refractory radionuclides.This assessment is no longer tenable when considering the results reported in this paper.

These results are presented here in two series:

- results of measurements performed on atmospheric particles around Chernobyl in the course of weeks following the accident,
- results pertaining to micro-scale laboratory experiments intended to modellizing the emission of radionuclides from the nuclear fuel.

Both kind of observations emphasize the complex nature of Ru emission from the damaged reactor and, particularly, its strong dependence upon oxidizing conditions of its surrounding atmosphere.

EXPERIMENTAL PROCEDURE

1. Determinations of radionuclides in the atmosphere in the zone of Chernobyl.

Around the Chernobyl reactor, aerosols were collected by filtration of air and the radionuclides collected were determined by γ- spectrometry .

2. Small-scale laboratory simulations

Particles of UO_2 (about 1 mg, enriched: 93% ^{235}U) have been irradiated for 3 x 7 hours by thermal neutrons under a flux of about 3 10^{11} n cm^{-2} s^{-1} (in the thermal column of the BR1 reactor, Mol, Belgium). In a first treatment, these particles were heated up stepwise to 2200°C in a graphite oven (from an atomic absorption spectrometer) under a flux of argon (1.2 L min^{-1}). Lower temperatures (<1000°C) were measured by a thermocouple while higher temperatures were determined by an optical pyrometer (Leeds & Northrup). The aerosols and vapours emitted in the process were collected onto quartz wool and counted for radionuclides activity (see figure 2). The UO_2 residue was also determined for its radioactivity content, together with the graphite oven.

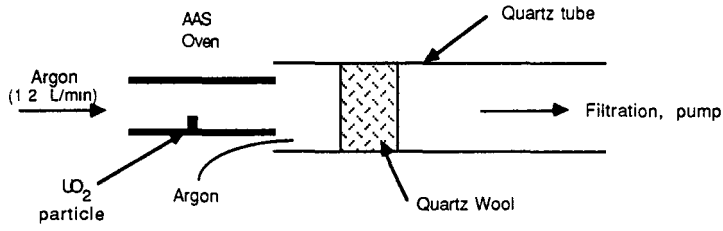

Figure 2. Scheme of the device used to warm UO_2 particles up to temperature of the order of 2200°C.

After this first thermal shock, the UO_2 particles were put into a quartz tube and inserted into a second oven where the temperature was stepwise increased up to 1200°C. In a first stage, the particles were swept by a flow of helium and the emitted radioactivity trapped onto quartz wool at the exit of the oven. After cooling down, the particles were swept by air and again submitted to a stepwise increase in temperature. In all cases, the temperature inside the oven was controlled by thermocouples.

The radioactivity collected onto the quartz wool was measured by γ - spectrometry on a Ge-Li detector (Philips APY 40 AIN) coupled to a 4096 - channel analyser (Canberra - 35 Plus). Results were corrected for radioactive decrease up to the end of the neutron irradiation.

RESULTS

1. Measurements in the zone of the accident.

Measurements were made on air particles collected in different areas around Chernobyl with emphasis on the enrichment of ^{103}Ru relative to ^{144}Ce (considered as a refractory element). The results presented here refer to a 50-day period after the reactor burst. It is particularly interesting to consider the period following the first nine days of intense release of radioactivity (see figure 1: determinations made at the KRI), where it appears that the aerosols are significantly enriched in radio-ruthenium. The reported values are rather erratic, supposedly because particles were sampled in various zones, irrespective of wind direction relative to the ractor. As a consequence, results were most probably influenced by resuspension of soil particles which blurred the direct contribution from the reactor.

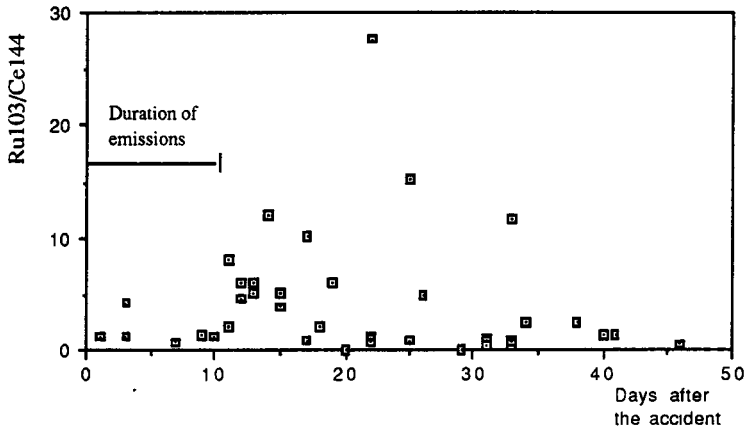

Figure 1. Enrichment of Ru relative to ^{144}Ce, in air particles collected around Chernobyl after the accident.

It is suggested that after nine days of intense emission due to the high temperature of the fuel, there was a rupture of the reactor vessel leading to a flow of the fuel into different substructures of the building. This led to a relative dispersion of the fuel, to a separation from the graphite moderator and to a subsequent cooling down. Moreover, during this stage, the fission products were brought into better contact with air. And this resulted in the oxidation of part of the Ru with enhanced emission of volatile oxide forms.

2. Laboratory micro-scale simulation of Ru emissions.

The experiments presented here under aim at demonstrating the pertinence of this emission scenario by means of micro-scale laboratory experiments.

Table I presents results obtained during a typical experiment in which an irradiated $^{235}UO_2$ particle was stepwise heated up to a temperature of 2000°C under a flux of argon. The duration of the heating stages is given. Different conclusions may be drawn from this table.

- Refractory elements such as Ce, La, Nb, Nd, Pm and Zr are very efficiently retained in the UO_2 matrice. The fraction released at 2000°C could be attributed to the uranium oxide losing some fraction of its matter as a consequence of the thermal shock.
- ^{99m}Tc and ^{132}I are short-living daughters nuclides of ^{99}Mo and ^{132}Te respectively: their behaviour closely mimic that of their parent nuclide.
- Despite of the temperature attained, a unusually high fraction of Xe (74 %) was retained in the UO_2 particle. In other experiments, the retained fraction was of the order of 20-25 % and this comes in contrast with the predicted 100% emission from the reactor.
- The case of radio-Ru is interesting: about 70-75 % of it is commonly retained in UO_2 particles brought to temperatures of 2000-2200°C for 2 minutes. It is suggested that the fraction which has been emitted at lower temperatures is attributable to the thermal shock and/or to a partial oxidation by traces of oxygen present in the argon. Note that the graphite of the oven could favour the retention of Ru given its reducing properties.

After this first operation aimed at scavenging all volatile radionuclides, the particle was put into a quartz oven and submitted to two subsequent mid-temperature treatments: under helium and under air. The results of one of these experiments are given in table II. In the case presented here, each temperature stage lasted for 2 hours. Results are presented in terms of percentage of radioactivity emitted from the UO_2 particle.

Table II. Percent of Ru released from an UO_2 particle heated up stepwise to 1200°C (2 h - stages) under a flux of He and *thereafter* under air.

Temper. °C (2h)	Under He	Under air
250	0.005	0.001
500	0.010	0.120
750	0.003	9.40
1000	0.001	44.35
1200	n.d.	42.38
Residue	100	2.40

When swept by helium, practically all Ru is retained in the UO_2 particle up to 1200°C, while under air almost one hundred percent is expelled. This demonstrates the very significant influence the atmosphere exerts in this process. Other experiments performed in much the same manner have systematically confirmed this influence.

CONCLUSIONS

This paper demonstrates the importance of the contact of oxygen in the emission of radioactive ruthenium from nuclear fuel. That this process occurs at relatively low temperatures (750 - 1000°C) emphasises the particular sensitivity of this radionuclide to redox conditions in the surrounding atmosphere. A parallel influence has already been found for cesium where oxidative conditions transform U(IV) into U(VI) and favour the formation of insoluble cesium uranates at temperatures of the order of 700°C (A.H. Al Rayyes *et al.* 1992).

In the case of a nuclear reactor accident with release of radionuclides, one of the major steps to be taken in order to reduce the emission of radio-Ru should be to avoid any contact of the fuel with air.

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ANNEX II.

Microscale deposition of air particles onto spruce twigs

ABSTRACT

Deposition of thin aerosols onto spruce twigs have been studied as a function of the speed of wind crossing the twigs. In the case of aerosol diameter less than 0.5 μm, *lowering* wind speed *enhances* deposition rates. This observation is undoubtedly important in the modelisation of the transfer of radioactive aerosols to forest ecosystems.

INTRODUCTION

The problem of deposition of air particles onto plant surfaces is a complex one as many mechanisms are involved in the phenomenon. On the one hand, there are macro- to mid-scale transports in which wind, atmospheric turbulence and eddies play the dominant role. On the other hand, there is a micro- to submicro-scale transfer onto surfaces which mainly depends on the characteristics of the particles (Lindberg *et al.*, 1982). Larger particles are deposited mainly by sedimentation and impaction, while thinner particles are mostly under the influence of their diffusion phenomena.

To the best of our knowledge, the majority of studies devoted to the deposition of particles onto plant material have addressed the first kind of mechanisms, i.e. the macro- to mid-scale transport toward plant cover. By doing so, they were able to stress the very strong positive effect of wind speed and of particles diameter on transfer efficiency (Belot *et al.*, 1974).

As far as thinner particles are concerned (diameter, say, < 0.5 μm), global air movements remain important during the macro-scale atmospheric transfer phase. But the last, micrometer-scale approach stage is primarily determined by diffusion and, in that case, the duration of stay of the particles in the vicinity of receptor surfaces is the main parameter to determine deposition efficiency. Obviously, in that case, wind speed exerts an unfavourable influence.

The present paper is an account of observations made in a wind-tunnel on the deposition of sub-micrometer particles onto spruce twigs. These experiments were undertaken with a view to understand better the general mechanisms governing the last stage of deposition of air particles onto plants during dry periods. Finally, this study was launched in order to define the of parameters of transfer of radioactive aerosols to forests ecosystems in the case of a serious nuclear accident.

AIR PARTICLES

As a general rule, size classes of air particles are best defined by their physical behaviour in the air: larger particles are ruled by gravitational settling and inertia mechanisms, while thinner particles are characterized by diffusion properties. Figure 1 illustrates this point by giving the mean displacement of spherical, smooth particles, of unit density, as a function of their diameter. Any other particle, whatever its physical diameter, shape, smoothness or density, which is observed to obey a given rate of displacement is said to have an "Equivalent Aerodynamic Diameter" (EAD) as given by the graph.

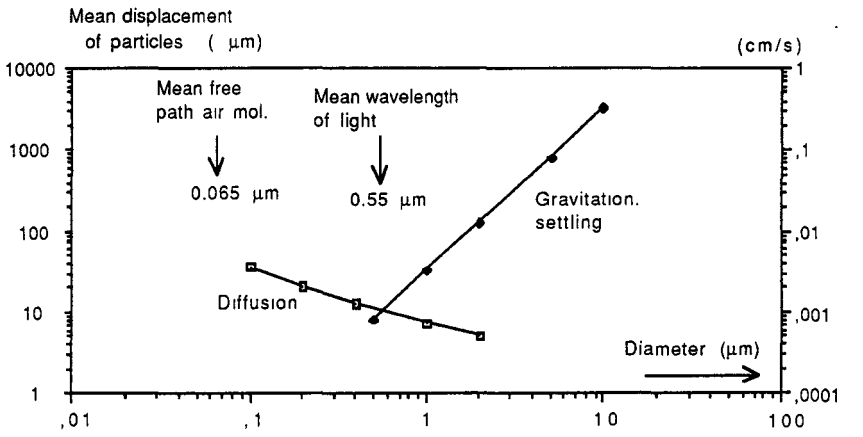

Figure 1. As a function of equivalent diameter, mean displacement (µm/s and cm/s) of particles in the air(values were calculated by the Stokes' law and by the Einstein's diffusion law).

It can be seen that below, say, 0.55 µm. diffusion dominates gravitational settling and becomes the main phenomenon to explain the movement of particles relative to air flux lines. Diffusion remains a micro-scale phenomenon and has to be taken into account only in the very vicinity of deposition surfaces. However, except for electrostatic forces, it is the dominant mechanism to determine the capture efficiency of small particles by surfaces. As a consequence, any parameter likely to influence diffusion will strongly affect the deposition efficiency of sub-micrometer particles.

It is interesting to notice that, except for gases, the radioactivity from Chernobyl which spread all over Western Europe in May 1986 was vehicled by sub-micrometer particles (Bondietti *et al.*, 1988). And although rain was generally the main factor to clean the atmosphere, significantly higher deposition rates of radioactivity were observed in forests as compared with pastures, for example, and this emphasizes the accumulation properties of tree canopies for such aerosols (Tobler *et al.*, 1988; Roed 1988; Roed 1988 b). Indeed, the accumulation of air pollutants by trees is a well-documented phenomenon (see for example Coenen *et al.*, 1987; Lindberg *et al.*, 1985; Witherspoon and Taylor, 1969; Belot and Gauthier, 1975).

EXPERIMENTAL PROCEDURE

Although only deposition onto single twigs is studied in this paper, the sizes of the wind-tunnel in which the experiments were performed are such that small trees may be submitted to deposition studies (length: 4.75 m - height: 2 m - width: 1.2 m). It is of a closed-loop model in order to be able perform radioactive contaminations with a minimal amount of radionuclides. Air was circulated by a battery of three superposed ventilators with adjustable power. Wind speed was measured by a Gill anemometer which is sensitive in the range 0.20 to a few m/s, provided its signal is estimated from the mean voltage given by the trace of a paper recorder. In further experiments with trees, smaller air velocity transducers will be used as their reduced size allows measurement to be made into the canopies.

Zinc oxide aerosols were generated in a quartz tube swept by a nitrogen flux. Metallic zinc pellets of about 0.3 g were gently heated up to temperatures of 800°C, well below the boiling point of the metal (907°C). This allowed the production of thinner aerosols at a steadier rate. At the very exit of the oven, the aerosols were mixed with air and flushed into the tunnel where they are dispersed by a fan. This resulted in an early oxydation of zinc into ZnO and prevented the recrystallization of the metal onto the colder parts of the generator.

Samples of aerosols were collected in the tunnel by filtration and by impaction (Aries Impactors) under isokinetic conditions. The mass-median aerodynamic diameter of the generated aerosols was about 0.2 - 0.4 μm. Analyses of impactor stages and filters gave thus mean concentrations of Zn in the air during the exposition of plant twigs which systematically lasted for 90 minutes, while the emission of aerosols lasted for about 10 minutes at the very beginning of the experiment.

Young twigs of spruce (Picea Abies Karst L.) were thus exposed to the aerosols under different wind speeds, systematically for 90 min. The twigs were then removed from the tunnel and the zinc oxide deposited onto plant surfaces was solubilized by washing with water at pH 2 for 15 min in an ultra-sonic bath. For some experiments, a distinction was made between the twig itself, and the inner part and the outer part of the needles (1/3 and 2/3 by length, respectively).

Zinc in the washing solutions was determined by flame atomic absorption spectrometry.

RESULTS AND DISCUSSION

In a first series of experiments, we determined the relative amount of aerosols captured by whole twigs. Results are expressed in mass percent of zinc retained by the twigs relative to the total mass of zinc which passed through the twigs during the exposure in the tunnel (the surface presented by the plant material to the flux of air was determined from xerox reproductions of the boughs). Figure 2 presents the results of a typical experiment where the fractions of zinc captured by twigs are given as a function of wind speed (fractions are expressed in 10^{-4}, *i.e.* the fraction of aerosols crossing the twigs which has been retained by the plant surfaces: mean of values measured on 10 twigs).

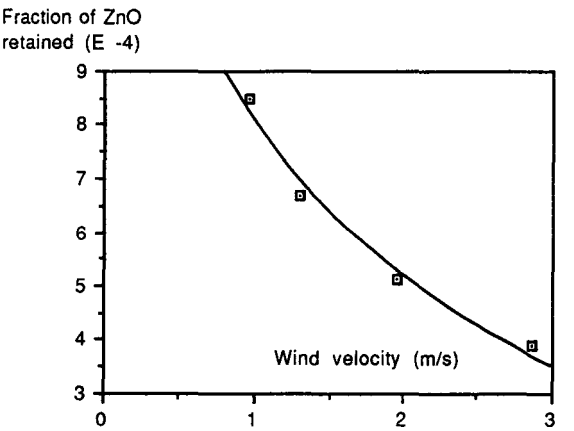

Figure 2. As a function of wind speed, fraction of ZnO aerosols retained by spruce twigs (fractions in 10^{-4})
(median EAD: 0.3 μm, mean concentration in air: 0.30 g/m^3)

Two features are noteworthy:
- the very low percentage of aerosols retained by the twigs,
- the strong influence of wind speed.

It is interesting to note that small deposition gauges were placed near the twigs. They were made of ordinary filter paper laying in the bottom of a cylindrical plastic cup (diameter: 10 cm - height: 10 cm). Determinations made on these paper surfaces show that deposition rates *increase* with wind speed and this demonstrates that deposition mechanisms are totally different in both twigs and gauge systems

This led us to examine further the influence of wind at lower speeds. Moreover, if lowering the speed of air exerts such an influence on deposition, microstuctures of twigs should play a significant role in blocating the movements of air in the very vicinity of the surfaces and deposition rates should very strongly depend upon the location of the surfaces relative to the center of the twigs. Experiments were performed at lower wind speeds, where Zn content was analyzed on twigs separated into three fractions:

- the branch itself,
- the first inner third of the needles,
- the two outer thirds of the needles.

Figure 3 presents the result of a typical experiment. The fraction of aerosols deposited is expressed in ppm.m^2/g (mass of Zn deposited onto the unit mass of dried plant material, divided by the total mass of Zn having passed through 1 m^2 during the whole experiment). Obviously, this is a logical way of expressing deposition yields when considering dissociated plant material; it is the *only* way when dealing with whole trees.

The influence of decreased wind speed is again confirmed but, more interesting, it appears that, at lower wind speed, the central parts of the twigs retain more aerosols than the outer fraction of the needles.

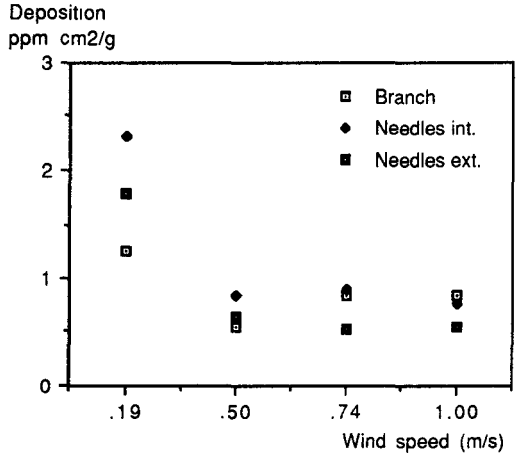

Figure 3. As a function of wind speed, deposition of ZnO aerosols onto different parts of a spruce twig (aerosol median EAD: 0.25 μm - mean concentration in air: 0.33 g/m^3).

It has to be noted that, although the surface presented by the branch itself is relatively low as compared to the surface of the needles, the accumulation of aerosols is far from being negligible.

Other experiments performed in the same way have confirmed this wind effect as well as the differential deposition into the twig structure. Note that these experiments are extremely sensitive to the progressive contamination of the wind tunnel which walls and equipment accumulate ZnO particles. This deposition seems to give way to an accretion of thinner particles into coarser ones which may be liberated during further experiments. In order to avoid this kind of interference, it is necessary to clean the atmosphere between each experiment by circulating air at high speed and eliminating these contaminant particles by a high-volume air particle sampler.

CONCLUSIONS

Wind speed enhances atmospheric turbulences over landscape features such as forests: as a consequence, the gross vertical transfer of air pollutants and, more particularly, of air particulates is enhanced on a macro-scale level. Turbulence also

enhances the capture of larger particles by soil and plant surfaces because, in this case, impaction is the main factor to determine the separation of particles from the air stream. Things are different for thinner particles where deposition onto surfaces occurs as a consequence of diffusion at a micro-scale level. In this latter case, deposition yields are enhanced at low wind speed.

In the modelisation of air pollutant deposition onto plant surfaces, such a phenomenon cannot be ignored. This is particularly true when dealing with thermo-generated aerosols such as those emitted by the reactor at Chernobyl: in this case, a great deal of the radioactivity was transported all over Europe into aerosols with EAD in the order of 0.2 μm.

Furthermore, it appears that deposition is also dependent upon the structure of plant surfaces, as was demonstrated by the different deposition yields observed in the dense internal parts of spruce twigs as compared with the more "open" structure of the external parts.

Further experiments are being undertaken in order to determine the particular deposition behaviour of fission products emitted by neutron-irradiated UO_2 samples heated up to temperatures of 2000 - 2500 °C.

ACKNOWLEDGEMENT

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ANNEX III.

The evolution of 'Hot Particles' in forest soils.

BEHAVIOUR OF RADIOCESIUM IN FOREST ACID SOILS

The type of soil and humus has to be considered primordial for the evaluation of the Cs availability and for its transfer dynamic. Previous studies made on the radiocesium distribution through the profile of a forest Brown Acid soil indicate that the intermediate layer OAh (organic and mineral particles intimately mixed) accumulates Cs in a more efficient way (in spite of its organic nature) than the deeper mineral layer. Our study on the physico-chemical parameters influencing Cs shows that humic compounds do not influence Cs retention. On the contrary, K interaction with the mineral layer of soil induces a clear increase of Cs fixation.

This last observation suggests that K-inducing collapse may be responsible for a higher Cs retention. In natural conditions, an original collapsed state of the mica structure of the OAh horizon would be beneficial for Cs fixation in this layer.

See, for example - Behaviour of radiocesium in forest multilayered soil, by Y. Thiry and C. Myttenaere, in: J. Environ. Radioactivity (to be published in 1993).

- Determination of the physico-chemical parameters which influence Cs availability in forest soils, by Y. Thiry, M. Vanhouche, P. van der Vaeren, S. de Brouwer and C. Myttenaere in: Seminar on "The dynamic behaviour of radionuclides in forests", SRPI (Sweden)-CEC (DGXI-D-3, DGXI-A-1) meeting, May 16-22, 1992, Stockholm, Sweden/

STUDY OF THE PHYSICO-CHEMICAL PROPERTIES OF ARTIFICIALLY THERMOGENERATED "CHERNOBYL-LIKE HOT PARTICLES".

PRODUCTION OF ^{160}TB HOT PARTICLES.

Terbium-160 (a γ-emitter) was choosen as a tracer of the evolution of UO_2 because, like uranium oxide, it forms a refractory, insoluble Tb_2O_3. Tagged uranium oxide particles were prepared by mixing 1.5 % (w/w) terbium (in the form of Tb_2O_3) with UO_2. Tb was previously irradiated for 3x7 hours by thermal neutrons under a flux of 3 10^{11} n/cm^2 s. The irradiated samples were then thouroughly mixed with UO_2 powder. The mixture was compressed into a pellet and brought up to temperatures of the order of 2300°C for vitrification. The pellets were then grounded and sieved to pass at 58 µm. This fraction was isolated (size comparable to real Chernobyl hot particles) and used for experimental work.

VERTICAL MIGRATION TEST

A preliminary study of the particles migration was realized in a column filled up with fine silt. A thin layer of radioactive particles was homogeneously deposited at the top of the column and covered by 0.5 cm of silt plus a fritted porous plate. Three leaching cycles were applied in 24 h intervals, each consisting in watering with 3000 ml of demineralized water, under a flow of 200 ml per hour.

The results obtained do not demonstrate any clear migration of the particles, in spite of the low size of the particular fraction (≤ 58µm) and in spite of the relatively high porosity of the column substrate. Only 0,01% of the total activity deposited onto the column surface had migrated and contaminated the first 3 cm of the column: 99.984% of the initial activity remained in the upper 7 mm.

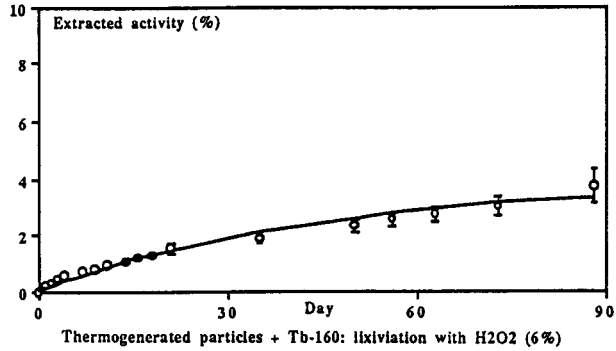
Terbium oxide being very slightly soluble and integrated into the particle matrix, any possible transfer of radioactivity would be due mainly to a physical migration of the particles themselves (swept along by the water flow). The absence of any migration observed in this case suggests that, after a major nuclear accident, particles from a damaged core should migrate toward sub-soil only in conditions of very high porosity of the surface soil layer (besides mechanical perturbations of the edaphic structure due to ploughing, for example).

IMPACT OF REDOX AND pH CONDITIONS

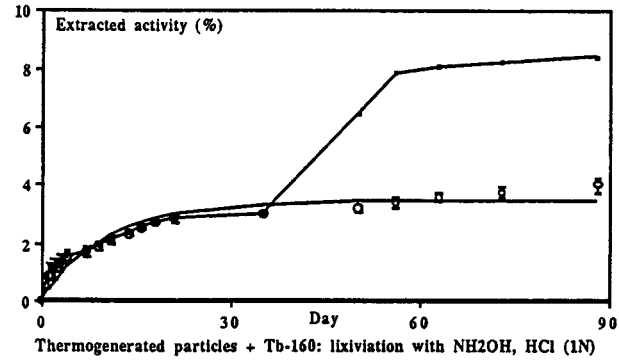
Particles prepared in much the same way (tagged with ^{160}Tb; size ≤58µm) were end-over-end shaken during 90 days: (i) under acidic conditions: in demineralized water at pH 1, (ii) under oxidizing conditions: in H_2O_2 (6%), (iii) under reducing conditions: in NH_2OH (1N) + HCl (10%). Samples of the particles (a few tens of µg - 10^5 to 2 10^5 Bq) were first thouroughly washed by distilled water. They were then introduced in batches of 20 ml of these solutions (3 repetitions). At given intervals (2 - 10 days), shaking was stopped and 15 ml aliquots were sampled after decanting. 15 ml of fresh solutions were then added before resuming extraction.

Results pertaining to these successive extractions are presented in the figures below: figures are given in terms of percentage of total activity extracted from the particles as a function of time (days).

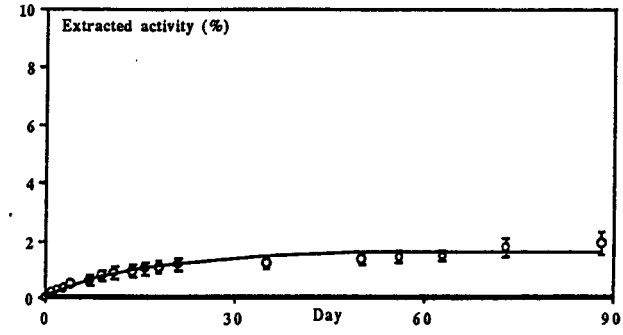
Thermogenerated particles + Tb-160: lixiviation with HCl (pH 1)



Thermogenerated particles + Tb-160: lixiviation with H2O2 (6%)



Thermogenerated particles + Tb-160: lixiviation with NH2OH, HCl (1N)



The observed values are very similar (very low standard deviation) for all kinds of treatments, except under H_2O_2. One curve shows a sudden disconnection, probably due to a desintegration of the structure of one of the particles (this hypothesis is supported by the restoration of the plateau, very parallel to the distribution of the other points after 56 days).

The general evolution of the curves has been modelized for each treatment, following a Mitcherlich equation:

$$Y = M^* (1 - \exp. (-t/b))$$

with M (maximal value at equilibrium in % of activity) and b (time: day)
which characterizes the rate of the ^{160}Tb lixiviation (in this model, 95% of the M value is reached after a time interval equal to 3b).

The table here under synthesizes the results obtained in accordance with this model.

	M (%)	b (d)
HCl pH 1	3,90 ± 0,25	45,5 ± 5,5
H_2O_2 6%	3,44 ± 0,10	10,31 ± 0,87
NH_2OH, HCl 1N	1,62 ± 0,06	16,04 ± 1,84

The results clearly show that particles made of an uranium oxide matrix vitrified at high temperatures are very resistant. Even under severe chemical conditions, the total lixiviation is very low: less than 4% in an acidic solvent, about 3,5% with an oxidizing reagent and less than 2% in reducing conditions.

It appears that oxidizing conditions are more aggressive since the plateau of the curve (M = 3.44%) is reached after a shortest delay: 3b ≅ 31 days. An acidic attack attains the same value but after a longer delay, the equilibrium being reached after only 3b = 135 days.

In conclusion, it appears that radioactive fallout in the form of "hot particles" represents a major ecological problem. Indeed, these particles seem to be resistant to lixiviation for long periods of time. As a consequence, the pool of radioelements included in these matrixes remains a long-term radioecological risk.

In the absence of physical countermeasures (like surface soil removal) and/or of migration with water flow, it appears that the radioactive decay of the radionuclides remains the only parameter likely to induce a significant decrease of the contamination level at the soil surface. The role and impact of the microflora on the behaviour of hot particles still remains to be thouroughly studied.

Progress Report

Contract: FI3P-CT920050 Sector: A25

Title: Cycling of cesium 137 and strontium 90 in natural ecosystems.

1)	Wirth	Bundesgesundheitsamt
5)	Impens	Faculté Sciences Agronom. Gembloux
6)	Belli	ENEA
7)	Feoli	CETA
8)	Nimis	Univ. Trieste

I. Summary of Project Global Objectives and Achievements

The main objective of the project is the study of the cycling of radiocaesium and radiostrontium within and between abiotic and biotic compartments of forests. For our investigations different coniferous and deciduous forests were chosen from different climate zones in Belgium, Germany, Italy, Luxemburg and Sweden. The experimental research emphasizes on parameters and mechanisms to understand and to quantify transfer processes of ^{134}Cs, ^{137}Cs and ^{90}Sr. The investigations of the participating groups cover the following topics:

- influence of soil parameters and soil types on the vertical migration of radiocaesium and radiostrontium in different layers of the soil,
- influence of soil parameters on the transfer rates from soil to different green understorey plants,
- analysis of uptake mechanisms for caesium and strontium in different habitats of mushrooms,
- influence of leaf- and needle-fall in dependency of weather conditions on the cycling of radiocaesium in forest litter,
- analysis of radiocaesium and potassium concentrations in different components of forest litter (leaves, needles, twigs and seeds),
- analysis of the retention of radiocaesium and nutrients from leaves and needles,
- influence of different ecological and climate situations on the leaf and litter production,
- identification of ecological understorey species groups to detect relations between contamination patterns and ecology,

- seasonal fluctuation of radiocaesium content in these different plant species, and
- dependency of the ecological characterization of forest ecosystems from soil parameters.

The sampling campaigns of the involved groups are in progress and will be continued. Based on these measurement results, including data obtained during the previous research work, several radioecological equations will be developed and their reliability will be statistically analysed: an equation describing the production and decomposition of forest litter as a source of radiocaesium for forest soil, a characterization of forest ecosystems by some special species of understorey vegetation due to their contamination levels and their ecology, a relation showing the seasonal fluctuation phenomena in radiocontamination patterns, and finally transfer equations for the uptake of radiocaesium and ^{90}Sr by different species of green plants and different habitats of mushrooms.
These equations, received by each group on different forest and soil types and under various climate conditions, will be compared to obtain the basic principles of migration and uptake mechanisms in forest ecosystems. Finally these different equations will be put together for modelling the behaviour of radionuclides in and between different compartments of forest soils and plants.

Head of project 1: Dr. Wirth

II. Objectives for the reporting period

The main objectives for the actual period are the behaviour of radiocaesium and ^{90}Sr in natural forest soils and their uptake by mushrooms and green plants. The emphasis of our investigations is the influence of soil parameters on the transfer rates from soil to plant of the above reported radionuclides. Additionally we study the effect of different concentrations of potassium and calcium within soil and plant on the activities of radiocaesium and radiostrontium in plants. Based on our results, we will develop transfer equations for different plant species and several soil types by using statistical methods.

III. Progress achieved including publications

To complete the data obtained in the years before, the collection of plant and soil material at the sampling site Hochstadt (pure Picea abies stand) is continued. Additionally we have selected two coniferous forests and one deciduous forest. Besides complete soil profiles, separated into the occurring horizons, we have sampled Fragaria vesca, Vaccinium myrtillus, Rubus fruticosus and Rubus idaeus. Also we have collected about 50 species of mushrooms, including the three groups symbiotic, saprophytic and parasitic fungi.
The analysis of the sampled material being dried, milled and weighted is in progress. After the γ-spectrometric measurements the samples are ashed and radiochemically prepared for β-counting. Soil parameters are determined by standard chemical

methods.
Together with the University of Trieste (Prof. Nimis), Italy, we study a mixed forest in the Carnic Alps, in the Eastern part of North Italy. The Italian group is engaged in the ecological characterization of this sampling site, while our institute does the analyses of the corresponding soil. A common publication is in preparation.

Publications:

Kammerer L., Hiersche L., Wirth E.: Uptake of radiocaesium by different species of mushrooms. Journal of Environmental Radioactivity, accepted 08.09.93.

Wirth E. et al.: Transfer Equations for Cs 137 for Coniferous Forest Understorey Plant Species. Science of Total Environment. 1993.

Wirth E., Kammerer L., Hiersche L.: Das Verhalten von Caesium 137 und Strontium 90 in Nadelwäldern - Ergebnisse 5jähriger Untersuchungen in Südbayern. Bundesanstalt für Fleischforschung, Kulmbach, 1993.

Head of project 5: Dr. Impens

II. Objectives for the reporting period

- To launch a new sampling campaign to collect bio-indicator fungi and plants, including soil samples, in various forest ecosystems in Belgium.
- To start experiments in a growth chamber to study the role of mycorrhizae in radiocaesium transfer.

III. Progress achieved including publications

The sampling campaign was carried out in six sites located in southern Belgium (about 300 soil, plant and fungus samples) and in the Grand-Duchy of Luxemburg.

Some of the samples were analysed. However, measurements had to be stopped because of some delay in the payment of EEC funds. For the same reason, the growth chamber experiment has been postponed until EEC funds are received.

We also conducted less expensive research, namely a bibliographical search and compilation on radioactive contamination in forest ecosystems and, particularly, in wild fungi.

We started analysing all data available on the contamination of fungi and we are currently working on a draft publication on that topic in collaboration with the Wirth and Nimis teams.

Head of project 6: Dr. Belli

II. Objectives for the reporting period

The main objectives for the period from January to August 1993 are following reported:

- selection of the experimental site;
- identification of the most suitable experimental protocol;
- samples collection;
- analysis of the samples.

III. Progress achieved including publications

Radiocaesium in forest canopy can be deposited on the forest floor by leaf-fall, stemflow and troughfall. One of the most important pathways for the input of contaminants in forest soil is the leaf-fall, the leaching of radionuclides from dead leaves and the litter decomposition.

The study of influence of this pathway on the cycle of radiocaesium in forest ecosystems is the objective of this project started in 1993.

The experimental site was established in a mixed forest in Tarvisio (Carnic Alps, Eastern part of Italy), located at 870 m above the sea level. The forest is characterized by Picea

excelsa (77 %) and Fagus sylvatica (17 %). The age of the forest ranges from 60 to 100 years. The mean annual rainfall is 1500 mm, and the mean annual temperature is 5 °C. The soil is classified as brown earth.

This area received significant deposition from the Chernobyl accident (about 40 kBq/sqm Cs-137). To study the feasibility of this research, a first estimation of the contribution of litter-fall was carried out in 1991. The result showed that during one year the input of Cs-137 attributable to this pathway is about 600 Bq/sqm.

In an area of about 100 m x 100 m, in which the presence of beech trees is generally higher than spruce trees, 9 subareas were selected to carry out the project. In each subarea one net and one trap with about a surface of 1 sqm each was located to assess the seasonal production of litter-fall and the seasonal input of caesium to the forest floor.

At the moment, two seasonal samplings of the litter-fall have been carried out and rainfall and air temperature are continuously recorded.

The main components of the litter (leaves, needles, twigs and seeds) have been assessed. Radiocaesium and potassium will be determined in each component.

To study the loss of weight and the retention of radiocaesium and nutrients by bagged leaf litter, 6 litter bags with inside about 20 g of Fagus leaves were placed on the surface of the forest floor in each subareas (total number of bags 54) of the experimental site. In two of these subareas, characterized by a higher presence of spruce trees, also 12 litter-bags with about 50 g of spruce needles were located to investigate the different caesium between Fagus leaves and spruce needles.

A weighed uniform litter sample was confined in bags of nylon mesh. The mesh size (about 2 mm) was selected as a compromise between providing maximum access and the forest fauna and minimising loss of small fragments from the bags.

Radiocaesium and potassium were determined in sub-samples of leaves and needles used to prepare the litter-bags.

Every two months from April to November, 11 bags are collected for the following analyses:

- weight determination;
- gamma spectrometric analysis for radiocaesium and potassium determinations;
- C and N content determinations.

Head of project 7: Prof. Feoli

II. Objectives for the reporting period

The experimental activity of the CETA group within the CEC project is to plan and to develop a mathematical model on forest dynamics for the evaluation of the seasonal leaf litter production. It will be linked with another model on radiocaesium circulation within the soil-to-plant system. This second model will be adapted from previous researches on radionuclides absorption in grasslands carried out by this group in the past years. The aim is to generate a comprehensive informatic tool to predict radionuclides circulation and re-availability under different ecological and climate situations.
The main objective for the reporting period is the field data collection that weekly records from April to October. The research is focused on ecophysiological and growth variables related to photosynthesis responses, and microclimate data recorded by fixed meteorological stations. Radioecological data connected with the litter decay will be furnished by the ENEA-DISP experimental plots (litter-bags). The results, structured in a data base system, will be used to calibrate the model parameters.

III. Progress achieved including publications

At the present moment, the field data collection is going on systematically, with weekly all-day recording campaigns. The ecophysiological parameters involved are:

- photosynthetic rate, transpiration, stomatal conductance on beech leaves (Fagus sylvatica L.) kept in different stages of development and environmental situations by means of an Infra-Red Gas Analyser (IRGA, ADC) that records the data in real time.

- leaf water potential on the same species, by means of a pressure bomb (PMS).

- soil water volumetric content, by means of a time-domain

reflectometry recorder (Trase system).

No model parameter calibration can be made now since that the seasonal collection will last till mid October. Partial data analysis is currently made to evidenciate correlation between ecological variables by means of advanced techniques of multivariate analysis. More than half thousand records have been collected up to this time.
Photosynthetic data are related to light intensity and temperature to calculate function response curves that will be in the growth model to predict the leaf production trough the vegetative season.
Water potential data are analysed in correspondence with the temperature daily profiles, demonstrating high correlation between these variables. This technique looks to be very promising in the future analysis. The interaction with other experimental results, i.e. the soil water content trend, the vegetation transpiration and the seasonal rainfall can set up the basis for the estimation of the water circulation and balance within the forest ecosystem. This factor is probably the key parameter in the radionuclides circulation and absorption, particularly in forests, where other ecological factors as macro- and micro-nutrients could be not so critical.

In the following figures two examples are illustrated from the field data analysis. Fig. 1 represents the photosynthetic response function versus light intensity, interpolated by experimental data with least squares technique. In this way the sub-model parameters for the light factor can be estimated. Fig. 2 shows the time-depending leaf water potential during one day. The point data have been interpolated by average values per hour to fit the corresponding response function.

Publications in the report period

Ardiani R., Belli M., Feoli E., Menegon S., Parente G., Sansone U., Scimone M., 1993. "The effect of fertilizers applications on ^{137}Cs uptake by different species of vegetation". Sci. Total

Environ. (submitted)

Ardiani R., Belli M., Feoli E., Menegon S., Parente G., Sansone U., Scimone M., 1993. "A mathematical model to predict ^{137}Cs uptake related to fertilization in grassland ecosystems". (in progress)

Ardiani R., Belli M., Feoli E., Menegon S., Parente G., Sansone U., Scimone M., 1993. "Analysis of the correlations between fertilization, ecophysiological variables and radiocaesium uptake in different grassland vegetation types". (in progress)

BEECH - photosynthesis rate

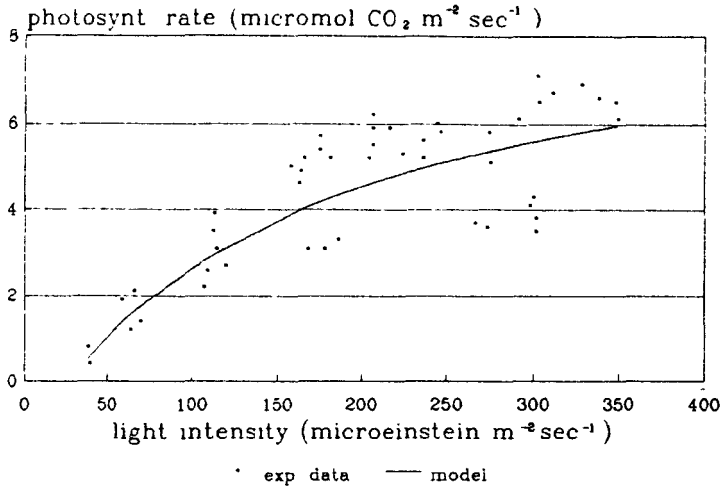


Fig. 1

BEECH - leaf water potential

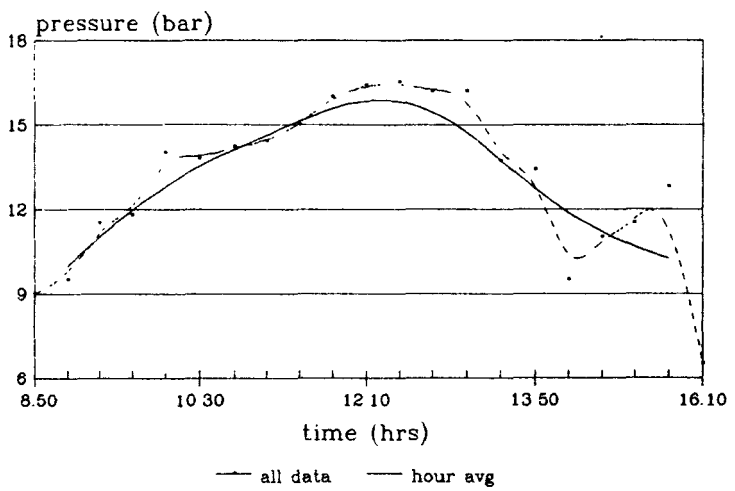


Fig. 2

Head of project 8: Prof. Nimis

II. Objectives for the reporting period

1) For each of the main ecological species groups identified during the previous research years, a limited number of species was selected. These species were sampled at regular monthly intervals to pursue a research started last year aiming at investigating possible seasonal fluctuation phenomena in the radiocontamination patterns.

2) Microreleves of understorey synusiae were carried out at the P.so Pura station in order to obtain a direct ecological characterization of the species, which could be used to detect the existence of possible relations between contamination patterns and ecology.

3) Using the preliminary results of the data analysis relative to point 2) soils were sampled under distinct synusiae in the forest understorey in order to obtain direct soil data for the ecological characterization of the synusiae themselves.

III. Progress achieved including publications

1) The first result relative to point 1) is that some species present clear fluctuation phenomena, and that for some of them there is probably a relation with the yearly rhythms of mycorrhization phenomena.
2) A very good relation was found between the ordination of species based on the microreleves and their contamination patterns.
3) Pteridophytes were found to substantially differ from Angiosperms in their contamination patterns, tending to accumulate radiocaesium in the leaf vacuoles.

The activity during 1993 can be summarized as follows:

1) Leafs, stems and roots of the following species: Abies alba, Anemone trifolia, Aposeris foetida, Athyrium filix-foemina, Cardamine enneaphyllos, Dryopteris austriaca, Dryopteris filix-mas, Fagus sylvatica, Gentiana asclepiadea, Gymnocarpion robertianum, Hupertia selago, Lycopodium annotinum, Oxalis acetosella, Phegopteris polypodioides, Picea excelsa, Polygonatum verticillatum, Polystichum aculeatum, Rosa pendulina, Vaccinium myrtillus, were sampled at the P.so Pura station in the months of May, June, July, August and September, an the activity of Cs-137 and Cs-134 was measured.

2) 150 microreleves of understorey synusiae were carried out at the P.so Pura station. The data were analysed by multivariate analysis to detect groups of species with similar ecology within the forest, and to obtain an ordination of species related to the main ecological gradients. The radioactivity data relative to a set of selected species were plotted on the ordination, revealing a very good correlation with the gradient. This allowed us to identify 5 main synusiae characterized by 5 different contamination patterns.

3) soils were sampled with scientists from Neuherberg under the 5 synusiae (see point 2); the material is under analysis at Neuherberg.

Publications:

NIMIS P.L., GIOVANI C. & R. PADOVANI 1986 - La Contaminazione de Cesio 134 e Cesio 137 neu macromiceti del Friuli-Venezia Giulia nel 1986. **Studia Geobotanica**, 6: 3-121.
NIMIS P.L., GIOVANI C. & R. PADOVANI 1988 - On the ways of expressing radiocesium contamination in plants for radioecologial research. **Studia Geobot.**, 8: 3-12.
NIMIS P.L., GIOVANI C., PADOVANI R., BERSAN F. & E. CEBULEZ 1988 - Utilizzo dei macromiceti come bioindicatori della migrazione del Cesio radioattiva negli orizzonti negli orizzonti pedologici. **Arch. Bot. Ital.**, 64 (3-4): 181-191.

NIMIS P.L. 1990 - Air Quality Indicators and Indices. The use of plants as bioindicators and biomonitors of air pollution. In: A.G. Colombo & G. Premazzi (eds): Proc. Workshop on Indicators and Indices, **JRC Ispra.** EUR 13060 EN: 93-126).

NIMIS P.L., TRETIACH M., BELLI M. & U. SANSONE 1990 - Individuazione di bioindicatori di radiocontaminazione. **Sicur. Prot** Roma, 21: 71-76.

NIMIS P.L., GASPARO D., GIOVANI C. & R. PADOVANI 1990 - Radiocontamination maps of macrofungi in north eastern Italy (Friuli Venezia Giulia) following the Chernobyl accident. **Gortania**, 11: 119-126.

NIMIS P.L., TRETIACH M., BELLI M. & U. SANSONE 1990 - The effect of microniches in a natural ecosystem on the radiocontamination of vascular plants. In: G. Desmet, P. Nassimbeni and M. Belli (eds.): "Transfer of radionuclides in natural and semi-natural environments". pp.: 84-93. Elsevier. London and New York.

GIOVANI C., NIMIS P.L. & R PADOVANI 1990 - Investigation on the performance of macromycetes as bioindicators of radioactive contamination. In: G. Desmet, P. Nassimbeni and M. Belli (eds.): "Transfer of radionuclides in natural and semi-natural environments". pp.: 485-491. Elsevier. London and New York.

NIMIS P.L. & E. CEBULEZ 1989 - I macromiceti quali indicatori di conatminiazione da cesio radioattivo nel Friuli Venezia Guilia. **Inform.Bot.Ital.**, 21, 1-3: 181-188.

NIMIS P.L., BOLOGNINI G. & C. GIOVANI 1993 - Radiocontamination patterns of vascular plants in a natural forest. - **Sci. Tot.Envir.** (submitted).

GIOVANI C., BOLOGNINI G. & P.L. NIMIS 1993 - Bryophytes as bioindicators of radioactive contamination. - **Sci. Tot. Envir.** (submitted).

Progress Report

Contract: **F13P-CT920058** **Sector: A25**

Title: **Radiation doses and pathways to man from semi-natural ecosystems.**

1)	Colgan	RPII
2)	Horrill	NERC
3)	Nielsen	Lab. Risø
5)	Veresoglou	Univ. Thessaloniki

I. Summary of Project Global Objectives and Achievements

1. Introduction

Consumption of foodstuffs produced from semi-natural ecosystems contaminated with man-made radioactivity can significantly contribute to the radiation dose of the general population and of critical groups in particular. Because of the long ecological half-life of artificial radioactivity in the natural vegetation and in the animal species which characterise such ecosystems, this dose can be delivered over a period of several decades.

Compared with agricultural ecosystems, there are limited data available on radionuclide cycling in semi-natural ecosystems. In addition, many of the factors controlling radionuclide uptake from these soils are not yet fully understood. This restricts the degree to which radiation doses from semi-natural ecosystems can be evaluated and allows limited predictive capability in the event of future nuclear accidents.

This research programme focuses on evaluating the importance of semi-natural ecosystems as a source of radiation exposure to man. One of the key parameters to be calculated is the ecological half-life, defined as the time taken for the concentration of a radionuclide in an ecosystem to be reduced by half, excluding losses due to radioactive decay. Because this parameter is likely to be different for different semi-natural ecosystems, an understanding of the mechanisms of soil-plant uptake of ^{90}Sr and ^{137}Cs, both in terms of transfer parameters and the interaction with competing ions such as Ca and K respectively, is also necessary.

Work on all these aspects of radioecology is being undertaken in a co-ordinated manner with a high degree of co-operation between participants. The aim is to provide more accurate estimates of the committed radiation doses from foodstuffs sourced from semi-natural ecosystems and to develop a predictive capability for future accidents. The data produced by the group will be essential in designing appropriate countermeasures to limit radiation doses to man in the future.

2. Plant Uptake Studies

2.1 Transfer parameters

The transfer of radionuclides from soils to plants is often defined in terms of transfer factors or transfer co-efficients. These terms were originally developed for studies of agricultural ecosystems but have proven to be less appropriate for semi-natural ecosystems. It is known that ^{137}Cs in soil exists in a number of fractions of different availability. Once the soil/soil water equilibria are established for the mobile fractions, additions to the more immobile fractions have large effects when transfer factors are calculated but are unlikely to significantly affect the radionuclide uptake by vegetation.

A new ^{137}Cs sequential extraction technique for soils with relatively low radiocaesium contamination has been developed using wet soils. This procedure is considered to be more representative of field conditions than existing techniques, most of which work with dried soils. The technique has been developed using very small sample weights of wet soils from New Galloway in Scotland and will now be applied to a selection of Irish peatland soils. The ^{137}Cs content of each extracted fraction will be correlated with plant uptake to see which fraction, or combination of fractions, provides the best estimate of plant concentration.

Because of the importance of animal products from semi-natural ecosystems as a source of radiation exposure to man, radionuclide levels in vegetation are often of more direct relevance than those in the underlying soils. In the western European semi-natural ecosystems, among vascular plants Calluna vulgaris usually contains the highest levels of ^{137}Cs and is often the dominant component of the vegetation. It is therefore a potentially useful bioindicator and can possibly be used to predict ^{137}Cs concentrations in other plant species.

A preliminary study at one site has shown that there is less variability in the ^{137}Cs content of Calluna vulgaris and Juncus squarrosus than of the underlying soils. The co-efficient of variation was 12% and 20% respectively for each of the vegetation species compared with 30% for the integrated deposition in soil and up to 60% in individual soil horizons. This work has now been extended to four other sites and the vegetation species sampled expanded to include Erica tetralix and Scirpus caespitosus. Data are presently being compiled and the predictive capability of plant-to-plant ratios and soil-to-plant transfer factors will be compared. Between site and within site predictions will be carried out and account will be taken of the results obtained from the sequential extraction correlations.

2.2 Cs:K and Sr:Ca relationships

In a series of glasshouse and field experiments using both stable and radioactive nuclides, the effects of K and Ca on the uptake of Cs and Sr respectively by a range of plant species is being investigated. The soils being studied have pH values in the range 3.5 to 7.0 and have an organic matter content ranging from 6% to 86%.

In general the Sr:Ca relationship has been found to be much stronger than that of Cs:K. For soils with similar organic matter content strontium uptake and pH have been found to be negatively correlated. Ca_{ex} in the soil and Sr in plant material are also negatively correlated. Ca^{++} and Sr^{++} in soil solution are found to be positively correlated. No relationship between Sr or Cs concentration in plant material and Sr_{ex} or Cs_{ex} in soils is observed.

3. Radionuclide Budgets

All participating laboratories are co-operating in a joint study on the inventory and distribution of ^{137}Cs and ^{90}Sr in a semi-natural woodland ecosystem in Sweden. Replicate sampling of each compartment was undertaken on 20th August 1992 and samples distributed among the participants for determination of soil physical and chemical parameters and radionuclide content.

The preliminary data for ^{137}Cs are summarised in the tables and pie-chart below. About 71% of the total ^{137}Cs activity is found in the soil compartment with up to 21% in the tree component, primarily in Picea abies which dominates the site. Above-ground

vegetation, fungi and animals represent about 1% of the total budget. In terms of radiation dose to man, the "foodstuffs" compartment is the most important and, given the high percentage of ^{137}Cs in the soil compartment and the known dynamics of the system, vegetation fungi and animals are likely to maintain high ^{137}Cs concentrations for many years.

Comparable data on ^{90}Sr in the ecosystem are presently being compiled. Activity levels in mosses lie in the range 19-71 Bq kg^{-1} dw with a mean value of 48 Bq kg^{-1} and no correlation has been found between the ^{90}Sr and ^{137}Cs content of individual samples.

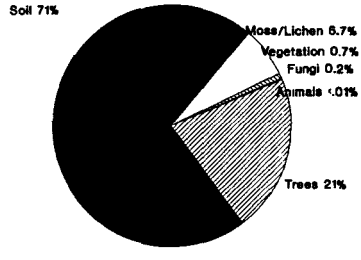
Distribution of ^{137}Cs within the Ecosystem

^{137}Cs ($MBq.ha^{-1}$)

Soils (0-10cm)	340
Trees	100
Moss/Lichen	32
Vegetation*	3.5
Fungi	1
Roe-Deer	6×10^{-4}
Moose	8×10^{-4}

**primarily vaccinium spp.*

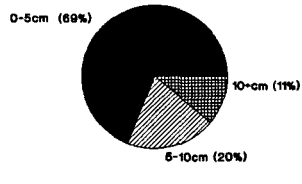
^{137}Cs ($Bq.\ m^{-2}$)



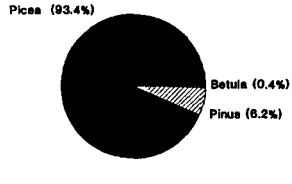
^{137}Cs in Tree Compartment (kBq per tree)

	Needles/Branch	Main Stem	Total
Picea abies	39.9 (80%)	10.1 (20%)	50
Pinus sylvestris	32.6 (89%)	4.2 (11%)	36.8
Betula pendula	4.3 (48%)	4.7 (52%)	9

^{137}Cs in soils ($Bq\ m^{-2}$)



^{137}Cs in Trees ($Bq\ m^{-2}$)



Soil characteristics (mean ± 1 SE)

Depth	pH	Organic matter (%)	Totals %	
			Ca	K
0-5	4.43±0.12	91.8±0.9	0.357±0.054	0.127±0.017
5-10	4.30±0.08	72.7±5.0	0.435±0.070	0.428±0.094
10+	4.48±0.10	32.2±13.9	0.725±0.079	1.460±0.312

Depth	Exchangeable (meq/100g).				C.E.C. meq/100g
	Ca	Ca*	K	K*	
0-5	17.29±2.67	17.85±3.58	2.85±0.39	2.05±0.26	139.9±3.9
5-10	7.05±1.20	-	1.27±0.15	-	132±10.4
10+	5.17±1.78	-	0.41±0.12	-	72.2±19.7

pH: *1:10 (w/v) using fresh soil*

Organic matter: *loss on ingition method*

Totals: *HF-$HClO_4$ digestion*

Exchangeable: *Ammonium acetate method 1m pH7.0*
Ca, K; dry soil
Ca, K*; fresh soil*

C.E.C. *$BaCl_2$ extraction*

4. Ecological Half-Lives

A study of radiocaesium distribution in soils at a peatland site in Ireland has allowed a comparison of the nuclear weapons and Chernobyl inputs. The data indicate that losses of ^{137}Cs from the ecosystem follow a two-compartment decay with effective half-lives of 8 and 22 years respectively.

One must distinguish between ^{137}Cs losses from soils and decreases in levels of radioactivity in vegetation. Chemical fixation in soils and the different rooting depths of various upland plant species must be taken into account. However, data on foodstuffs produced in the Faeroe Islands shows that the relative reduction with time of the ^{137}Cs from the Chernobyl accident is much faster than that observed for nuclear weapons fallout. It is possible that this observed difference merely reflects the two-compartment decay observed in Irish soils and, with time, the rates of reduction for the two sources of contamination will become identical.

Further work is necessary to refine the ecological half-life estimates for each of the ecosystems being studied. These data will be used in conjunction with national statistics on foodstuff production and consumption rates to evaluate the importance of semi-natural ecosystems as a source of radiation dose to man in different Member States. The data provided from the plant uptake studies presently underway will improve the capacity to predict concentrations in vegetation and foodstuffs thereby permitting better estimates of committed doses in the event of future accidents involving radionuclide deposition.

Head of project 1: Dr. Colgan

II. Objectives for the reporting period

1. Continue time series evaluation and ecological half-life estimations of radiocaesium uptake by peatland vegetation, including an investigation of site variability in plants and soils.

2. Continue studies on the utilisation of plant-to-plant ratio systems. Compare and evaluate such a system relative to conventional transfer parameters.

3. Complete a study on the uptake and transfer of ^{137}Cs to flesh, relative to concentrations in faeces and rumen, of individual animals culled from a flock of free-ranging red deer *(Cervus elaphus)*.

4. Undertake a study to determine the residence time of ^{137}Cs in peatland soils.

III. Objectives for the next reporting period

1. Calculate ecological half-lives of ^{137}Cs in peatland vegetation and in mountain sheep for comparison with existing data on ^{137}Cs residence time in soils.

2. Compile data on foodstuffs consumption to be used for committed dose calculations.

3. Provide data on ^{90}Sr content of peatland vegetation for ecological half-life calculation.

4. Complete a study using pollen traps installed on honeybee hives as a means of integrated sampling of peatland vegetation over the forage area of the hive.

IV. Progress achieved including publications

Soil-Plant Transfer of ^{137}Cs

Investigation of site variability in radiocaesium estimations for soils, plants and transfer parameters has been completed and the data have been used to assess the efficiency and accuracy of site comparisons of radiocaesium contamination and of soil-plant

transfer. The results clearly show that there is less within site variability in the ^{137}Cs content of plants compared with soils, and hence between site differences can be identified much more efficiently by vegetation sampling than by collecting soil samples. Between site comparisons using plant-soil transfer parameters are possible but authors should quote confidence intervals with their data; we demonstrate the use of accepted methods and adapt an existing method to account for the log normal ratio distribution which our data revealed.

Figure 1 shows a plot of the log of the plant soil concentration ratio against the log of soil radiocaesium activity. A highly significant regression line has been fitted to the data. This relationship is contrary to the theory on which concentration ratios are based, but is found to hold true for the two peatland plant species studied and for both ^{137}Cs and ^{40}K. It is proposed that the relationship be expressed as a transfer function:

$$C_{plant} = AC_{soil}^{1+b}$$

where C_{plant} is the plant concentration, A is the antilog of the intercept on the regression on the y-axis, C_{soil} is the concentration in soil, and b is the slope of the least square regression. Such transfer functions could be used to compare sites more effectively than would be possible using simple transfer parameters.

Work on the comparative value of plant-to-plant ratios relative to plant-to-soil ratios has been completed. Figure 2 illustrates the finding that there was less variability associated with plant-to-plant ratios than with conventional ratio systems at the same site, confirming the validity of the concept. The same data clearly illustrate that the actual range of a ratio value is not indicative of the statistical variability (compare the range of ratio values for Calluna:soil activity, with that of Calluna:soil deposition, then look at the respective coefficients of variation), and shows graphically that ratios can be misleading and that appropriate statistics are particularly important for ratio values.

Ecological half-lives

A study on the distribution of ^{137}Cs in adjacent peatland and mineral soils has allowed short- and longterm losses from the peatland area to be evaluated. The integrated ^{137}Cs deposition in the mineral soils due to nuclear weapons testing showed good

Figure 1

Plot of the log of the plant soil concentration ratio against the log of soil radiocaesium activity.

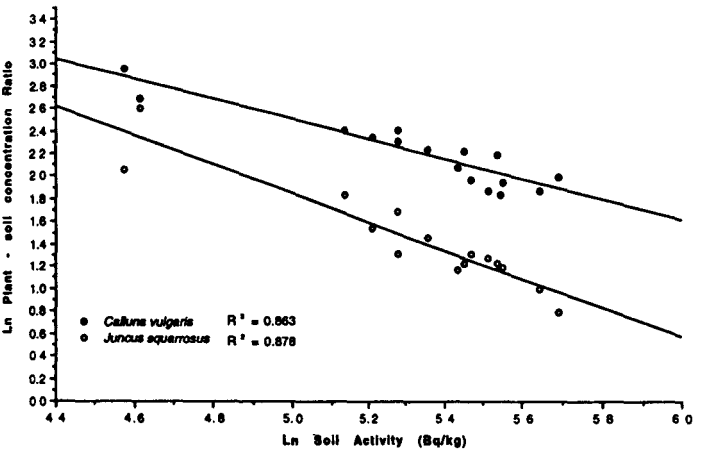
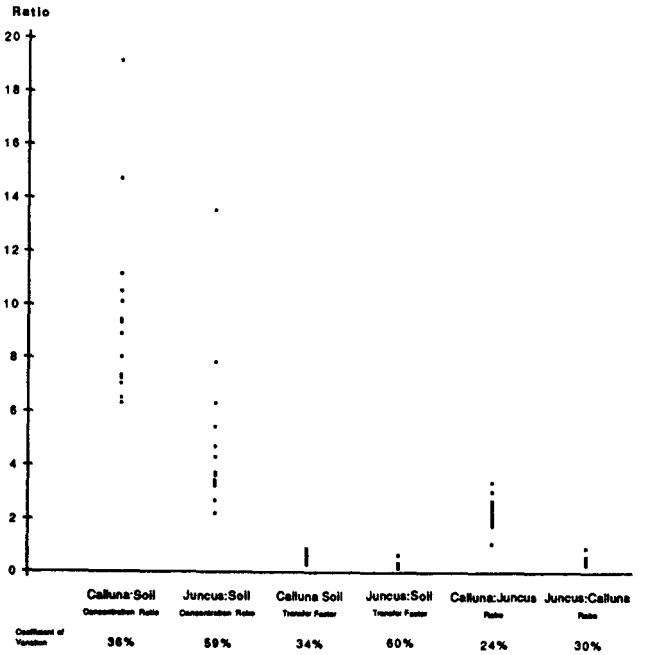


Figure 2

Dot-plots of plant to soil concentration ratios, soil transfer factors, and plant to plant ratios. Associated coefficients of variation are presented beneath each data set.



agreement with a value predicted from previous correlations with rainfall. Analysis of variance showed a highly significant ($P<0.0001$) difference in the ^{137}Cs retention capacity of both soils. Significant depletion of ^{137}Cs, representing 62.5% of the nuclear weapons fallout and 47% of the Chernobyl deposition, was observed. A two-compartment decay with effective half-lives of approximately 8 and 22 years, respectively, was calculated.

Time series data for ^{137}Cs in peatland vegetation from four sites for the years 1988 through 1993 have been completed. The radiocaesium content of sheep flocks grazing three of these sites has also been followed since 1989. Figure 3 illustrates the data from one of these sites and includes the early data points for 1993. All of these data are presently being evaluated and ecological half-lives for ^{137}Cs will be calculated for comparison with the values for residence times in soil.

Animal Studies

Tables 1 and 2 show results of an investigation of radiocaesium intake, absorption and excretion by a flock of red deer (Cervus elaphus). Forty two individual animals were culled from a flock grazing a peatland site. The altitude of slaughter, sex and age, together with radiocaesium concentration in rumen contents, kidney and faecal pellets of each animal were recorded. Highly significant correlations were found between the altitude of slaughter and the ^{137}Cs concentrations in rumen, kidney and faeces of the animals.

Table 1

Pearsons Product Moment correlation coefficients for altitude of slaughter, ^{137}Cs concentrations in kidney, rumen and faecal samples from 42 red deer.

	Altitude	Kidney	Rumen	Faeces
Altitude	1.00			
Kidney	0.530**	1.00		
Rumen	0.423*	0.796**	1.00	
Faeces	0.498**	0.854**	0.853**	1.00

** = significant at the 99% confidence level,*
*** = significant at the 99.9% confidence level.*

Table 2

Pearsons Product Moment correlation coefficients for altitude of slaughter, and ^{40}K concentrations in kidney, rumen and faecal samples from 42 red deer.

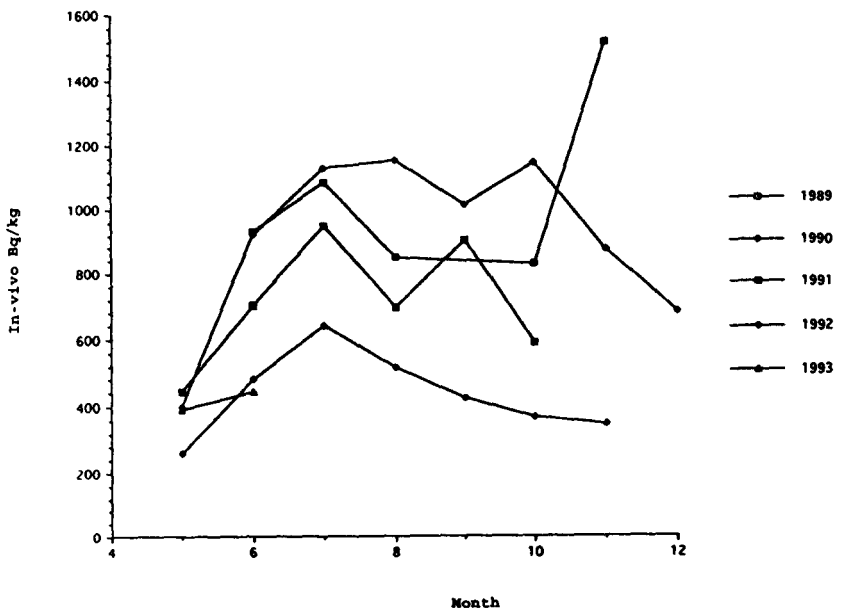
	Altitude	Kidney	Rumen	Faeces
Altitude	1.00			
Kidney	0.207	1.00		
Rumen	-0.089	0.022	1.00	
Faeces	-0.134	0.0.021	0.432*	1.00

* = *significant at the 99% confidence level.*

The ^{137}Cs concentration in the flesh (approximated by kidney) showed highly significant correlation with both intake (rumen) and excreta (faeces). This confirms that the relationship, previously shown by the same authors for sheep and faecal samples, also holds true for this animal species. Results for ^{40}K show a correlation between intake and excreta, but no correlation with flesh concentrations were observed. These findings confirm a difference of behaviour of these two radionuclides.

Figure 3

Radiocaesium (^{137}Cs and ^{134}Cs) content of ewes



Publications

1. McGee. E.J., Synnott, H.J. & Colgan, P.A. (In prep.) Ingestion and metabolism of ^{137}Cs and ^{40}K by red deer (Cervus elaphus) on peatlands.

2. McGee, E.J., Keatinge, M.J., Synnott, H.J. & Colgan, P.A. (In prep) Assessment of between site differences in radiocaesium and ^{40}K using plants, soils, transfer parameters and transfer functions.

3. McGee, E.J., Synnott, H.J. & Colgan, P.A. (submitted) Variability in soil deposition, plant concentrations and transfer parameters of ^{137}Cs in peatland soils.

4. McGee, E.J., Synnott, H.J. & Colgan, P.A. (1993) A new method for prediction of radiocaesium levels in vegetation: evidence from Irish uplands. *J. Environ. Radioactivity 18* 53-70.

5. Rafferty, B., McGee, E.J., Colgan, P.A. & Synnott, H.J. (1993) Dietary intake of radiocaesium by free ranging mountain sheep. *J. Environ. Radioactivity 21* 33-46.

6. Colgan, P.A., McCann P., McGee, E.J. & McAulay, I.R. (1993) Short- and long-term losses of ^{137}Cs from peatland soils. *Irish Journal of Agricultural and Food Research 32:* 37-46.

7. McGee, E.J., Synnott, H.J. and Colgan, P.A. (1992) Availability of radiocaesium from Chernobyl and bomb fall-out to peatland vegetation in Ireland. *Proceedings of IUR Workshop on soil-plant transfer of radionuclides* Madrid 1-3 June 1992.

Head of project 2: Dr. Horrill

II. Objectives for the reporting period

1) To collect and analyze species of fungi collected in UK habitats.

2) To investigate a site in Scotland for the occurrence of stable strontium in plants and soil.

3) To develop a sequential extraction technique for radiocaesium in soils.

4) To develop an analytical method for ^{90}Sr and cross-calibrate with other laboratories.

5) Assist other members of the group with gamma analysis and provide advice on nutrient element analysis.

6) Provide information on food product with gamma analysis and provide advice on nutrient element analysis.

7) Participate in joint group study on site near Uppsala.

III. Objectives for the next reporting period.

1) To analyse some organic peats from Ireland and other sites using the sequential extraction method described above.

2) To assess whether there is correlation between any of the extracted fractions of ^{137}Cs and ^{137}Cs concentrations in heather growing on the peat.

3) To assist in the compilation of a joint report on the Group study of a semi-natural woodland at Uppsala, Sweden.

4) Assist other members of the Group with gamma analysis and provide advice on nutrient element analysis.

IV. Progress achieved including publications

1) Fungi were collected from the English Lake district and the New Galloway region of Scotland. ^{137}Cs concentrations ranged from 60-500 Bq kg^{-1} in the Lake District and from 1700 - 50 000 at the New Galloway site. Soil concentrations were of

the order of 160 and 650 Bq kg^{-1} respectively. The data has been passed to Prof. Karl Johanson for inclusion in a European data set.

2) Two abandoned mining areas sited on the Ardnamurchan peninsula were visited. Stable strontium levels were determined in soil and a range of plant species from each site. Extractable strontium varied from 0.25 to 15 mg per 100 g and plant concentrations from 2.0 to 370 ug g^{-1}, the highest concentration being found in *Saxifraga hypnoides*. The data is available to group members and the sites appear suitable as stable strontium study areas.

3) Published methods for sequential soil analysis of ^{137}Cs have used small amounts of highly contaminated soil. It was felt that it was important to develop a method that could be used for organic soils from moderately contaminated ecosystems so that the results were more applicable to field situations. The method developed uses 50g of fresh organic soil and has the following sequential extraction stages - two water extractions, an ammonium acetate pH7 extraction, a 0.04M hydroxylamine hydrochloride in 25% v/v acetic acid extraction and a 7M nitric acid extraction. All samples extractants are evaporated to dryness in order to concentrate the activity for counting. The residual material is also counted so that an inventory of soil components associated with ^{137}Cs can be constructed for each soil layer (fig 1).

4) The analytical method for ^{90}Sr is now operational and results are in good agreement with those from the Risø laboratory.

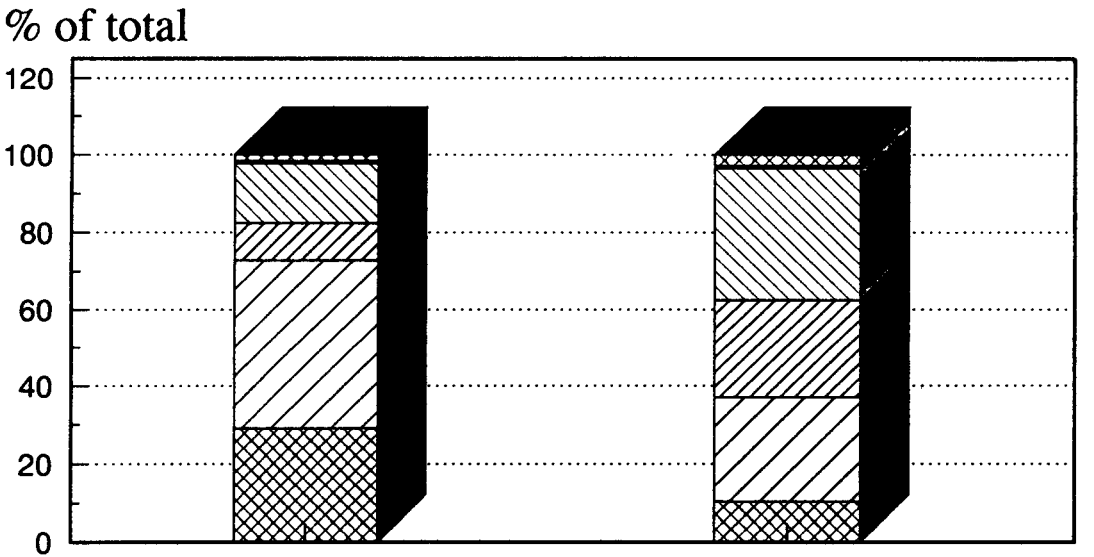
5) Gamma analyses have been completed for soils from the Uppsala investigation and advice supplied to the laboratory at Thessaloniki on soil nutrient analysis.

6) Bryophyte data from the Uppsala study has been evaluated and presented at a group co-ordinating meeting at Thessaloniki in May 1993.

Figure 1

Sequential extraction of 137-Cs

New Galloway Sites



	0-5cm	5-10cm
Remainder	29.08	10.34
7M nitric	43.72	26.60
Hydrox	9.54	25.49
Ammonium	15.35	34.03
Water 2	0.63	0.59
Water 1	1.68	2.95

Head of project 3: Dr. Nielsen

II. Objectives for the reporting period

The objectives for the reporting period have been 1) to participate in the joint field investigation in a forest area in Uppsala, Sweden, with relatively high Chernobyl radionuclide deposition and carry out Sr-90 analyses on samples from this area, 2) to participate in Sr-90 intercomparisons with the other participants, 3) to carry out a sampling campaign in the Faroe Islands to continue the time-series of data on radiocaesium for the determination of ecological halflives and 4) to prepare the background for an improved assessment of the doses to members of the public in the member states from radiocaesium in semi-natural ecosystems.

III. Objectives for the next reporting period

The objectives for the next reporting period will be 1) to finalise the Sr-analyses of samples from the Uppsala field investigation and carry out an intrepretation and reporting of the data, 2) to summarise the Sr-90 intercomparison data including those of a Russian laboratory with which RPII are co-operating, and 3) to analyse samples from the sampling in the Faroe Islands and calculate ecological halflives for radioceasium and 4) to carry out an assessment of the doses from semi-natural ecosystems. Furthermore, when individual sub-projects are finalised, the results will be written up for publication in scientific papers or reports.

VI. Progress achieved including publications

Uppsala field investigation

All project participants took part in the Uppsala field sampling which occurred on 28 August 1992. The objective of this investigation is to make a detailed study of the inventories of radiocaesium and radiostrontium in one hectare of Swedish coniferous forest and determine the distribution of activity in the different ecosystem sub-compartments. The Cs-137 deposition in the area amounts to about 60 kBq per square metre. Risø National Laboratory is doing Sr-90 analyses on vegetation (lichens, mosses, fungi and other plants) as well as meat. Up to this point the results on Sr-90 in mosses and lichens are available and these data have been compared with the results of Cs-137 in the same samples. Due to the small quantities of sample material available, it was decided to bulk the samples

according to species. A total of 14 samples of mosses and lichens were analysed for Sr-90. Analyses of variance of the results were carried out, but no significant differences were found between neither species nor locations within the sampling area. Table 1 gives the mean results for each species and the grand mean.

Table 1. Results of Sr-90 concentrations (Bq kg^{-1}) in mosses and lichens from the Uppsala sampling given as mean values for each species.

Species	Sr-90 (Bq kg^{-1} dm)
Polytrichum	33
Mixed bryophytes	71
Hylocomium splendens	64
Pleurozium schreberi	51
Sphagnum	43
Cladonia	27
Mean	48

A comparison between the concentrations of Cs-137 and Sr-90 in the samples is shown in Figure 1, which does not show a strong association between the levels of the two radionuclides. A correlation coefficient of 0.51 is calculated from the data, and a regression analysis gives a small positive slope which is significantly different from zero. In practice all the radiocaesium in the samples originates from the Chernobyl accident, and the intercept calculated from the regression analysis supports the assumption that this is also the case for Sr-90. The average ratio between the concentrations of Sr-90 and Cs-137 in the samples is 0.8%.

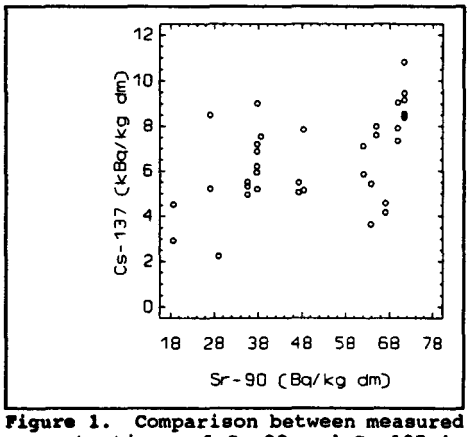


Figure 1. Comparison between measured concentrations of Sr-90 and Cs-137 in samples of mosses and lichens from the Uppsala sampling.

Sr-90 intercomparison

Only two of the participating laboratories are presently doing Sr-90 analyses (RPII and Risø). For intercomparison purposes several samples (dry milk and vegetation) have been distributed among the laboratories for both Cs-137 and Sr-90 analyses. In addition, results from IAEA intercomparison samples were included in this project since they were already available. The results of the Sr-90 intercomparison is shown in Table 2.

Table 2. Intercomparison of Sr-90 analyses showing concentrations (Bq kg^{-1} dm) in IAEA samples in addition to samples derived from this project. Where multiple determinations are made, the table gives mean values ± 1 sd.

Sample	RPII	Risø
IAEA 134, Cockle flesh		4.9±0.3
IAEA 135, Sediment		117±5
IAEA 373, Grass	1131±35	1390±70
IAEA, Soil	94±2	100±5
RPII, Dry milk	< 3	0.63±0.03
ITE, Bracken		121
ITE, Heather		44

The results could indicate a bias of the Sr-90 results between the two laboratories. This will be more clear when the remaining results from RPII become available. Furthermore, since RPII considers to involve a Russian laboratory in their Sr-90 analyses, that laboratory will be included in the intercomparison.

Faroese sampling

In June 1993 a sampling campaign was carried out covering the Faroe Islands. The sampling focused on soil, grass, milk and lamb at a number of locations. Due to local conditions this year it was not possible to cover all the planned locations. The samples will be analysed for both radiocaesium and radiostrontium.

The results are used to investigate the behaviour of Chernobyl radiocaesium relative to that from the weapons fallout. The general trend known from previous results is that the relative reduction with time of the radiocaesium in the Faroese ecosystem from the Chernobyl accident is much faster than that from the weapons fallout. This is apparent from Figure 2 which shows the levels of Cs-137 in Faroese milk since 1962. It is expected that the rates of reduction with time become identical for the two sources of contamination. These trends are followed from the above sampling programme.

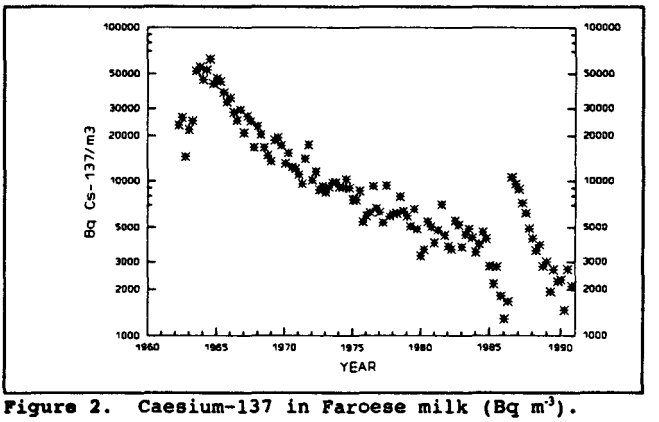


Figure 2. Caesium-137 in Faroese milk (Bq m^{-3}).

Doses from semi-natural ecosystems

During the previous contract period for this project, the doses to members of the public from radiocaesium in semi-natural ecosystems were assessed. From this work it became clear firstly, that only little information is readily available in the member states for this assessment and secondly, that there is a high variability between the member states of the relative importance of radiation doses from semi-natural ecosystems (50% in Sweden vs. 1% in the UK). For that purpose a detailed questionnaire has been drawn up and circulated to the project participants on consumption rates of the most important produce from semi-natural ecosystems as well as from usual food products. The food products from semi-natural ecosystems comprise game, fresh water fish, mushroom, lamb and goats cheese and the usual food products comprise milk and milk products, cheese, grain products, potatoes, vegetables, fruits, meat and marine fish.

The collective doses will then be calculated from the levels in the food, the effective ecological halflives and the consumption rates.

Head of project 5: Dr. Veresoglou

II. Objectives for the reporting period

1) To find out if any relationship exist between Sr and Ca or Cs and K concentrations in plant tissues among plant species grown in the same growth medium.
2) The effects of the physicochemical characteristics of the soils and the additions of Ca or K on the uptake of Sr or Cs by plant species.

III. Objectives for next reporting period

Two long term experiments are in progress from which data are not yet available. In the first experiment pots (40 dm^3 volume) were filled with soils differing considerably in the various physicochemical characteristics. A mixture of seeds of 10 pasture species was shown in each pot in April 1992. After the above ground vegetation was clipped twice, Sr at the rate of 220 mg/pot or Cs at the rate 668 mg/pot were applied by spraying on the soil surface on the late November 1992.
In the second experiment, six lysimeters 1.25 x 0.80 x 0.35 were filled using six of the nine soils used in the previous experiment. A mixture of seeds 10 species were sown and Cs-137 at the rate of 154 KBq/lysimeter was sprayed at the soil surface on November 1992 after the above ground vegetation was clipped.

IV. Progress achieved including publications

1. Relationship between Sr (or Cs) and Ca (or K) concentrations among plant species frown in the same growth medium.

i. Glasshouse experiments. Different plant species were grown in monocultures in pots (1 dm^3 volume) in different soil treatments. In the first experiment three soils were used. Sr was applied at the rate of 67 mg/pot. Above ground plant material was harvested twice and analysed for Sr and Ca concentrations. In the second experiment one soil was used with and without p addition. Sr was applied in the rate of 25 mg/pot. Shoot and roots were analysed for Ca and Sr concentrations.

ii. Field experiment. In an upland pasture two levels of N addition were combined with two levels of P in a block randomised experiment. In order to detect the root activity of the co-existing plant species in different depths in the soil, Sr and Cs were applied at the depths of 5 and 15 cm in May 1992 and 1993. Four weeks after application, the above ground plant material was harvested and Sr, Cs, Ca and K were measured in the component species. For 1993 the plant material was harvested also from the quadrants in which Sr and Cs were applied 13 months

earlier. In the glasshouse experiments a positive linear regression between Sr and Ca concentration in plant material was found in all soils treatments, harvest or part of plant examined. In the field experiment and in the case of Sr and Cs application at 5 cm depth the same relationship was evident for Sr and Ca concentrations but not for Cs and K concentrations.

2. The effects of soil type and the additions of Ca or K on the uptake of Sr or Cs by plants.

Six soils were used in a pot experiment (1 dm^3 volume) in which *Trifolium repens* was grown. For each soil a combination of 3 Sr levels x 3 Ca levels or 3 Cs levels x 3 K levels was used. Above ground material was harvested twice for measuring Sr or Cs, Ca and K. At the end of the experiment soils were analysed for Sr or Cs, Ca and K for both the extractable ($CH_3COOONH_4$) form or in the soil solution. The results of the experiment showed that:

a). Sr or Cs concentrations in plant material were not correlated with extractable Sr or Cs of the soils.

b). Sr concentration in plant material is inversely correlated with extractable Ca in the soil.

c). The addition of K reduced Cs concentration in plant material while that of Ca increased in some cases or reduced in others the Sr concentration of plant material.

d). The Sr, Ca, Cs and K concentration in the soil solution varied considerably in the different replicates of the same treatment. Variation in Sr concentration correlated positively with Ca concentration.

Publications

1. Veresoglou, D.S., I. Tsialtas, N. Barbayiannis & G. Zalidis. Caesium and strontium uptake by two pasture plant species grown in organic and inorganic soils (sent for publication).

2. Veresoglou, D.S., N. Barbayiannis & G. Zalidis. Report of the Progress of the work of AUT.

3. Veresoglou, D.S., G. Zalidis, N. Barbayiannis, S. Kalpakis & E. Batianis. Relation of Sr and Ca concentrations in tissues of plant species grown in different soils in pots (in prep).

4. Mamolos, A.P., G.K. Elisseou & D.S. Veresoglou. Spatial and temporal patterns of root activity of pasture species grown on acid inorganic soils in Northern Greece.

Progress Report

Contract : **F13P-CT920013a** **Sector : A26**

Title : Studies of methods for the rehabilitation of soils and surfaces after a nuclear accident (RESSAC).

1)	Maubert	CEA-Cadarache
2)	Sandalls	UKAEA
3)	Vandecasteele	CEN/SCK Mol
4)	Vallejo	Univ. Barcelona Fac. Biologia Vegetal
5)	Förstel	KFA
6)	Gutierrez	CIEMAT
7)	Arapis	Univ.Athens
8)	Kirchmann	Faculté Sciences Agronom.Gembloux

I. Summary of Project Global Objectives and Achievements

En dépit des mesures prises quant à la sûreté des réacteurs, un accident majeur n'est pas à exclure sur une installation type PWR. De ce point de vue, il convient d'étudier avec la plus grande attention le comportement des radioéléments pouvant être relâchés en cas d'accident afin de mettre au point des techniques permettant d'en limiter les conséquences sanitaires et de rendre le plus rapidement possible le site à une vie normale.

C'est dans ce but que les différents partenaires ont convenu du programme scientifique suivant :

1/ Expériences analytiques
- étude de la solubilité des "particules chaudes",
- étude comparative des facteurs de transfert aux plantes du Cs et du Sr, en solution ou générés à haute température.

2/ Expériences in situ
- test d'efficacité du Tapis Végétal Décontaminant,
- test d'efficacité des polysaccharides comme inhibiteur de la resuspension et du transferts aux plantes,
- essais de défoliation non létale pour la décontamination des forêts,
- mesures des facteurs d'interception des cultures vis à vis des aérosols en cas de pluie.

3/ Expériences globales en lysimètre
- prélèvements de monolithes de sols européens représentatifs,
- acquisition in situ de données climatiques,
- expériences en lysimètres disposés dans un bâtiment dit "Installation RESSAC" permettant leur contamination avec des aérosols générés par un four dit "POLYR"simulant ceux qui seraient produits par un accident nucléaire.

Head of project 1 : Dr. Maubert

II. Objectives of the reporting period

La période considérée, qui va de mai 92 à septembre 93, a été essentiellement axée sur l'achèvement de l'installation "RESSAC" et des dispositifs annexes (dispositif de contamination)ainsi que sur la mise en place des lysimètres.
Parallèlement le programme prévoit un certain nombre d'expériences de terrain portant sur l'utilisation des polyacrylamides dans les techniques de décontamination ainsi que l'étude de défoliants non létaux.
Par suite de problèmes essentiellement financiers, les premières contaminations de lysimètre prévues pour le printemps 93 n'ont pu avoir lieu et un programme de substitution a dû être mis en place.

III. - Progress achieved

III.1 - Aménagement du bâtiment spécifique RESSAC (Bât 186 du CE Cadarache)

La période considérée a été particulièrement importante du point de vue de l'aménagement du bâtiment. Les points à signaler sont les suivants :

- Réception de la plus grande partie des travaux à savoir : génie civil, électricité, plomberie, voies d'accès, ventilation nucléaire et climatologie. Restent à terminer ou à mettre au point l'appareillage de radioprotection, les équipements spéciaux de la serre N° 4 (portique, raccordement du four POLYR...), régulation du potentiel hydrique des lysimètres, chateau de plomb, transport de sources et mise en chaud (équipement vestiaire, masques...)

- Réception et utilisation de la table élévatrice, qui est un véhicule sur coussin d'air destiné à la manipulation et à la mise en place des lysimètres. Cet ensemble de 24 tonnes, charge comprise s'est révélé très facile d'emploi.

- Amélioration des performances du four POLYR. 16 essais, dont 6 en actif, ont été effectués. Par une adaptation de la forme et de la masse du creuset en graphite les performances en température ont été améliorées. La température atteinte (2 950°C) est maintenant tout à fait représentative du corum en fusion. Un étalonnage de la température du four a été effectué par fusion d'UO_2 (Tf = 2 830°C).

- Equipement des lysimètres. Sur les 7 lysimètres en place 3 (Belleville, Barcelone et Harwell) sont actuellement équipés de sondes tensiométriques et de sondes de température. Ces dernières sont placées à 30 et 120 cm de profondeur ; les sondes tensiométriques quant à elles sont à 30, 90 et 120 cm.

La régulation du potentiel hydrique est en cours de mise en place. Le prototype ayant donné satisfaction, une commande en série a été lancée.

III.2 - Prélèvement des lysimètres

Après la démonstration de faisabilité effectuée en juin 91 sur le site de Tricastin, 5 autres lysimètres ont été prélevés et installés dans le bâtiment. Sont donc actuellement en place :

2 sols Tricastin
1 sol Belleville
1 sol Barcelone
1 sol Harwell
1 sol Julich
1 sol Mol

Le tableau suivant en résume les caractéristiques :

Nationalité	Dénomination (lieu exact de prélèvement)	Date d'extraction	Date de mise en place	Type de sol
F	Tricastin (Lamotte du Rhône)	30/06/91	15/06/92	Alluvial calcaire
F	Belleville (Briare)	23/05/92	11/06/92	Alluvial sableux
B	Mol (SCK/CEN MOL)	21/08/92	28/08/92	Podzol
D	Julich (MERZENHAUSEN)	24/08/92	01/09/92	Loess
UK	Harwell (WELLESBOURNE)	22/09/92	28/09/92	Sol brun
E	Barcelone (BARCELONE)	20/10/92	26/10/92	Terra rossa

Parallèlement à ces prélèvements, ont été mis en place sur les sites de prélèvement des ensembles d'acquisition de données permettant d'obtenir, sur une année complète, l'évolution des gradients d'humidité et de température en profondeur ; ces données ne sont en effet pas disponibles auprès des services météorologiques.
Les sites de Belleville, Mol, Barcelone, Julich et Harwell ont ainsi été équipés et les informations acquises.
Les campagnes de mesures sont actuellement en cours sur les sites de Belleville et Harwell.

III.3 - Cultures sur lysimètres

Le programme initial prévoyait pour le printemps 1993 des contaminations de culture dans le bâtiment Ressac. Pour des raisons essentiellement financières ce programme n'ayant pu être suivi, un programme de remplacement a été élaboré en accord avec nos partenaires européens ; il porte essentiellement sur deux points :

- Etude des conditions de croissance des plantes en serre.

Afin de tester le réalisme des conditions de cultures, une même variété de blé (variété ARBON) a été semée en janvier 93 sur 4 lysimètres (Belleville, Harwell, Mol, Barcelone) avec les techniques propres à chaque pays ; toutes les opérations d'entretien (plan prophylactique, engrais, apport d'eau...) ont été menées par le personnel de Ressac. Le climat dans chaque serre a été conforme aux consignes affichées. La moisson effectuée en juillet/août a été faite par chaque partenaire. L'exploitation des différentes mesures effectuées sur les récoltes nous amène à estimer le rendement pour le lysimètre français à :

62,5 quintaux à l'hectare pour le grain

72,2 quintaux à l'hectare pour la paille,

rendements tout à fait comparables à ceux que l'on peut obtenir pour une culture de plein champ.

Pour que la structure du sol soit conservée et afin d'apporter un complément d'azote, les quatre lysimètres ont alors été plantés en trèfle (vériété espagnole "*Alejandria o Bersin*"). Ce trèfle sera retourné sur place pour servir d'engrais vert.

- Contaminations expérimentales.

Le programme de substitution comporte pour 1993 deux tirs effectués avec le four POLYR.

Le premier a eu lieu le 1er septembre. Le four POLYR comportait la charge standard de 16 éléments inactifs plus deux radioéléments ^{134}Cs et ^{85}Sr. Les différents partenaires européens ont apporté différents supports devant être contaminés. La participation française a été, outre l'équipement du dispositif et le fonctionnement du four, la mise en place de 500 filtres en acétate de cellulose (∅ 4,7 cm, 0,4 mm) afin d' estimer le taux de dépôt d'aérosols et leur forme physico-chimique.

Ce premier tir sera suivi d'un second avant la fin de l'année.

III.4 - Essais in situ

Les essais in situ effectués sur le terrain expérimental du CE Cadarache ont porté essentiellement sur les problèmes de défoliation non létale des plantes pérennes (forêts, vignes...). En effet, pratiquée rapidement après un dépôt radioactif accidentel, cette technique permettrait :

- d'empêcher l'incorporation des radionucléides dans les plantes et éviterait ainsi la contamination des récoltes futures,
- de récupérer une grande partie du dépôt et de contribuer ainsi à la dépollution du site.

Cinq produits ont été testés, à savoir l'éthephon (CIBA-GEIGY) le diméthipin (UNIROYAL), l'amonium gluphosinate (ROUSEL-UCLAF), le triclopyr (DOW) et l'hydroxyde de cuivre.

Il apparaît que la dose d'emploi a une très grande influence sur l'efficacité de cette technique et que les quantités à utiliser soient très différentes d'une espèce à l'autre.

A condition d'être appliqué en quantité suffisante, le dimethipin, habituellement utilisé pour le défanage des pommes de terre, donne les meilleurs résultats. Des chênes défoliés au début de l'été ont montré une reprise 1 à 2 mois après traitement.

Le rythme relativement lent de ces expérimentations est imposé par le cycle des saisons

Par ailleurs, il a été mis au point une technique rapide de dosage du ^{90}Sr dans les sols et les végétaux utilisée notamment pour la mesure des échantillons ramenés du site de Tchernobyl. Cette méthode repose sur l'extraction du strontium grâce aux éther-couronne suivie d'une reprise à l'eau. Le comptage est effectué en scintillation liquide. Le rendement de l'opération est déterminé grâce à l'ajout de ^{85}Sr.
La méthode est rapide, mais la sensibilité est limitée à 20 Bq. kg^{-1} . Elle peut trouver une application immédiate en période accidentielle pour effectuer rapidement des mesures à niveau relativement élevé.
Une campagne d'intercomparaison avec les méthodes employées par nos partenaires européens a été lancée.

IV. Publications

- **A.JOUVE, H.MAUBERT, E.SCHULTE** - Biomechanical removing of contamined soil : a field experiment.
Proceeding of the Symposium on Waste Management, TUCSON, (Arizona) 1-5 March 1992

-**M.KANEVSKY, U.KISILEV, Ph.FACHE, J.TOUCHE** - Cartographie orientée vers la décision dans les situations d'urgence et post-accidentelles.
Séminaire commun SFRP-FS "Impact des installations nucléaires sur l'environnement" Fribourg (Suisse) 15-18 sept. 1992

- **B.T.WILKINS, R.ALEXAKHIN, B.J.HOWARD, H.MAUBERT** - Development of strategies for agricultural countermeasures.
Sciences of Terrestral Ecology (soumis à l'éditeur).

- **J.M.QUINAULT et all.** - Enseignements tirés des études de transfert de radionucléides dans les sols et les cultures de la région de Tchernobyl.
Séminaire commun SFRP-FS "Impact des installations nucléaires sur l'environnement" Fribourg (Suisse) 15-18 sept. 1992

- **Ph.PICAT** - Les conséquences de l'accident de Tchernobyl : la contamination de l'environnement. Journée SFEN Paris 19 nov.1992

- **A.JOUVE, H.MAUBERT** - Présentation de quelques techniques de réhabilitation des sols après un accident nucléaire.
Séminaire commun SFRP-FS "Impact des installations nucléaires sur l'environnement" Fribourg (Suisse) 15-18 sept.1992

- **A.JOUVE et all** - No lethal defoliation to impair the foliar uptake of all-out radionucleides by forest trees.
Séminar on the dynamic behaviour of radionuclides in forest. Stockolm (Sweden) May 18-22,1992

- **N.MARY**- Etude de l'utilisation d'un polyacrylamide dans la réhabilitation des sols et des surfaces après un accident nucléaire.
Mémoire de 3ème année en vue de l'obtention du diplome d'Ingénieur des Techniques de l'Equipement Rural

Head of project 2 : Dr. Sandalls

II. Objectives for the period May 1992 to May 1994

These were as follows :

(i) to extract a soil monolith from a suitable location in the United Kingdom to the specification of the Eurosoil RESSAC project, and install it at the RESSAC facility at CE Cadarache.

(ii) to install and maintain an AXONE data acquisition unit at the original location of the monolith and establish a telemetric link with the RESSAC facility at CE Cadarache.

(iii) to design and implement a technical programme regarding the use of the lysimeters within the RESSAC facility.

A further objective arose in June 1993 when problems regarding the satisfactory completion of the RESSAC facility occurred, effectively delaying the completion of the original agreed technical programme. This objective was to design and implement a substitution programme of work for the lysimeters to complete the substitution programme of work for the UK lysimeter and to re-commence the original technical programme (delayed from 1993) devised for the UK lysimeter.

III. Progress achieved including publications

1. Extraction of UK Soil Monolith

The UK monolith was extracted from a site at the experimental farm of Horticulture Research International (HRI), Wellesbourne, Warwickshire in central England. This site provided a soil which has been well characterised over a period of some twenty years. The soil is an arable Brown Earth of the Wick series (Grade A), and had recently been used for wheat prior to extraction of the monolith.

The choice of the HRI institute at Wellesbourne as a sampling site, also allowed provision of convenient amenities, such as an electrical power supply and communications. These were utilised during excavation of the monolith. These services were also required for the AXONE data acquisition unit that was to be installed at the site. This unit would provide data of soil moisture and temperature to recreate natural conditions within the soil monolith, installed in a lysimeter in the RESSAC building at CE Cadarache.

Excavation of the soil monolith, using the technique described by Moutier, RESSAC Note 44/90, CE Cadarache (October 1990), took place over some ten days in September 1992. The monolith, of dimensions 1.8 m X 1.8 m X 1.75 m depth was then transported in a steel tank to CE Cadarache. A photographic record of all stages of the extraction was made.

During December 1992 and January 1993, the AXONE data acquisition unit was installed and commissioned successfully at HRI Wellesbourne. Measurements of soil moisture and temperature have been made continually since then.

Climatic data for the HRI Wellesbourne site has been sent to CE Cadarache. This data, in the form of long-term monthly averages for parameters such as rainfall, is required for simulation of the Wellesbourne climate for the UK lysimeter, now installed within the RESSAC building.

2. Technical Programme of Work on the UK Lysimeter

In December 1992, a meeting of all collaborators of the Eurosoil-RESSAC programme of work to be carried out on the lysimeters from each of the participating countries, i.e. Belgium, France, Germany, Spain and United Kingdom.

For each lysimeter a schedule for crop-sowing, contamination of crops by radioactive aerosol using the POLYR facility, and crop-harvesting was drawn up within an overall programme. A protocol for treatments to be applied to their lysimeter during 1993 was produced by each collaborator.

At the beginning of March 1993, the UK lysimeter surface was divided into two equal halves by a stainless steel plate, part A on the eastern side, part B on the western side. Each half was then cleared of weeds and cultivated. A variety of ryegrass (Morenne) was sown on part A while the wheat variety (Arbon), standard to all lysimeters, was sown on part B. The application of fertiliser and pesticides to each of the crops followed the treatments protocol for the UK lysimeter.

Sampling trays were inserted into the soil to a depth of 6cm, three on each side of the lysimeter. These were to provide a convenient means of sub-sampling crops and soil after contamination by radioactive aerosol.

In comparison with the other lysimeters which were sown at different times this was to produce wheat crops at different growth stages at the time of contamination. Contamination of the UK lysimeter was scheduled for May 1993.

The UK lysimeter provides an exception in that grass instead of wheat was sown on the second half. This alteration reflects better the agricultural situation and use of pasture land within the UK.

The growth of the crops was monitored by RESSAC staff at Cadarache who also irrigated the lysimeter by hand, stimulating the long-term average rainfall of the Wellesbourne area. Air temperatures and humidity were regulated according to average values for the original UK and German sites, since these two lysimeters shared the same section of the RESSAC greenhouse.

Part of the UK wheat crop was observed to fall down or "lodge" during the growth period. A sub-sample of a few plants was taken to try and determine the cause of this. Preliminary investigations have suggested that either the cause was a fungus or a fertiliser effect. However, the crop recovered to some extent before maturity.

The ryegrass was cut at an early growth stage to promote tillering out to ensure good cover of the lysimeter surface. A further cut was made later in the summer.

A delay in completion of the RESSAC facility has now prevented the original programme devised for 1993 from being carried out. It is intended to repeat this programme during 1994. In the meantime a substitution programme for 1993/94 has been devised (see section 4). The harvest of the wheat and ryegrass crops grown on the UK lysimeter was carried out during 17th and 18th August 1993 as part of the substitution programme (see section 4).

3. Intercomparison of Analysis for Radiocaesium and Radiostrontium

An intercomparison exercise is taking place in order to ensure the comparability of measurements made in the different national laboratories. Standard samples of plant material and soil, provided by RESSAC staff, have been distributed to each participant for analysis in their national laboratory.

Analysis of ^{134}Cs, ^{137}Cs and ^{85}Sr in the standard samples has been completed at Harwell Laboratory. Analysis of ^{90}Sr is currently being undertaken. Results will be sent to E. Schulte (Cadarache) for co-ordination upon completion of ^{90}Sr analysis.

This intercomparison exercise has been incorporated into the substitution programme for 1993/94 (see section 4).

4. Substitution Programme for the UK Lysimeter in 1993/94

At a meeting of the collaborators of the Eurosoil-RESSAC programme at Cadarache on June 7th and 8th 1993, it was deemed necessary for each participating country to devise a substitution programme of work for 1993/94. This was brought about by the postponement of the original programme due to delays in completion of the RESSAC facility at Cadarache.

A schedule for the proposed substitution programme for work on the UK lysimeter was sent to E. Schulte in June 1993.

The main areas of this substitution programme involve :

(i) a characterisation of crops harvested from the UK lysimeter in August 1993.

(ii) the establishment of a "parallel" field plot at HRI Wellesbourne to compare crops of clover and ryegrass grown in the field to those grown on the lysimeter in the RESSAC greenhouse.

(iii) to carry out soluble tracer experiments with ^{90}Sr and ^{137}Cs to provide information on the interception and retention of these radionuclides by wheat and ryegrass. The soluble traces will provide "worst case" conditions with 100% bioavailability of the radionuclides. These data can then be compared with results from the intended application of the POLYR aerosol to the UK lysimeter in 1994.

The wheat and ryegrass crops were harvested by S. Baker on 17th and 18th August 1993. An evaluation of the yield of the wheat crop and its root development is currently being made. The yield of ryegrass is to be recorded also. Samples of these crops will then be processed and a full multi-element analysis for essential major and minor elements will be made.

After harvesting of the wheat crop, a variety of clover "Alejandria" (provided by the Spanish collaborators) was sown on part A. This clover was sown at a standard rate (14 g/m^2) on all other lysimeters. P K fertiliser (supplied by Germany) was applied at a rate of 76 g/m^2. This was necessary to match conditions for sowing of clover at the "parallel" field plot established at HRI Wellesbourne.

On 19th and 20th August 1993, a "parallel" field plot was established at HRI Wellesbourne. An area identical to that of the lysimeter surface has been cultivated. Ryegrass and clover seeds have been sown at densities equal to that for the UK lysimeter, using similar fertiliser application rates also. The area is fenced to prevent disturbance by animals or machinery.

At the end of July 1993, four polypropylene containers of dimensions 60 cm X 60 cm X 40 cm depth were filled to a depth of 30 cm with the UK soil type obtained from HRI Wellesbourne.

On July 29th,ryegrass was sown on two of these lysimeters, with wheat being sown on the other two. Varieties of crop, sowing rates and fertilisation conditions were identical to those used on the UK lysimeter at Cadarache. These containers are located in a glasshouse at Harwell Laboratory. Irrigation of the lysimeters is being made by hand according to long-term average rainfall data for the Wellesbourne site. It is intended to contaminate all four lysimeters with tracers when the growth of the wheat crop reaches the end of shooting stage, probably in Autumn 1993.

Head of project 3 : Vandecasteele C.M.

II. Objectives of the reporting period :

In preparation of the RESSAC - EUROSOIL programme, a large sandy podzol monolith had to be extracted in summer 1992 and sent to Cadarache (CEA) to be housed in the RESSAC greenhouses. In 1993, a spring wheat culture had to be installed on the soil. The plants were supposed to be exposed to radioactive aerosols generated by the POLYR furnace at a given development stage.
Due to delays in the installation of the required equipment in the greenhouse, the contamination was postponed by one year and a substitution programme had to be adapted. In agreement with the CEC representatives, it was decided to make use of the uncontaminated material present on the lysimeter to check for the realism of the growth conditions recreated in the greenhouse by measuring the production yield and plant mineral content and to gather preliminary information of the behaviour of aerosol radiocaesium and -strontium in the European soils selected.

III. Objectives of the next reporting period :

The experimental programme, initially foreseen for 1993, will be carried out in 1994. Spring wheat will be sown again and exposed to the deposition of radioactive aerosols at a given development state. The interception factor will be estimated in relation to the standing biomass and ploughing, as a countermeasure, will be performed on one half of the Belgian lysimeter. The contamination of the plant aerial parts and translocation to the grains will be measured.
In the meantime, as part of the substitution programme, the wheat samples from the 1993 cropping will be analysed and the dynamic behaviour (adsorption - desorption and extractability) of caesium and strontium, as aerosol particles and ionic forms, will be investigated in batch for the 6 European soils already present in the greenhouse.

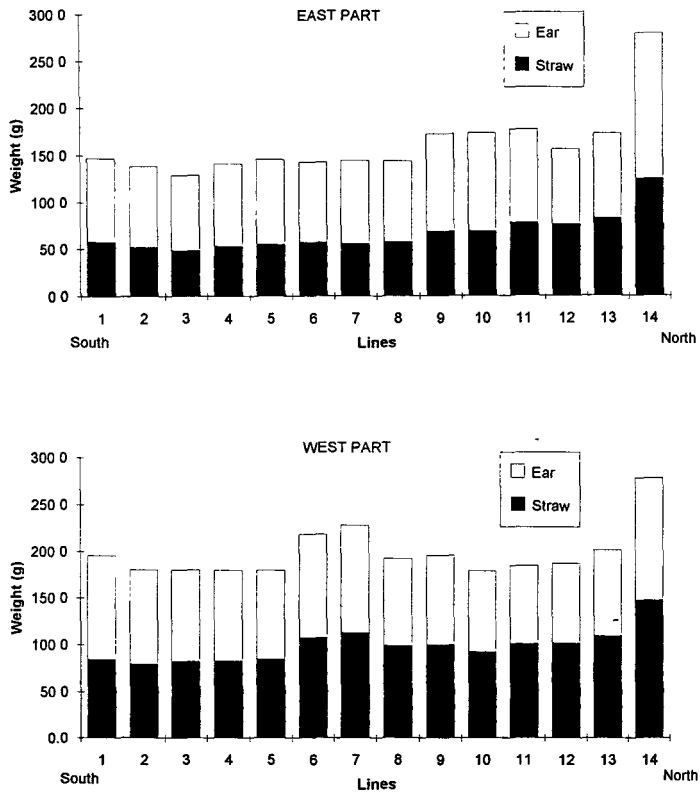
IV. Progress achieved :

Different types of soil originating from various European countries were selected for the RESSAC - EUROSOIL programme, representing a large range of soil properties. In this framework, the CEN/SCK was asked to provide a typical sandy podzol.
In order to reproduce in the RESSAC greenhouse at Cadarache, where the lysimeters had to be housed, climatic conditions as comparable as possible to those that are specific for the region where the monolith originates from, several relevant parameters were measured over more than one year, from winter 91 until winter 92. Therefore, the zone of the monolith was instrumented with tensiometers and temperature sensors at different depths until 1.2 m below the ground level. The collected data were transferred to the CEA-Cadarache by modem.
In our podzol soil, the water potential appears to be rather constant over the whole profile and throughout the seasons, at a value of about -0.15 bar. The temperature at 30 cm depth increased from 8°C in winter 1991-92 to 17°C in summer 1992. The temperature gradient with depth was lower in winter (1.5 °C/m) than in summer (3 °C/m) between 30 and 120 cm.
In addition to the temperature and water profiles in the soil, other climatic parameters (air temperature and humidity, rain frequency and intensity, sunshine frequency and intensity) were measured locally or obtained from the national meteorological stations network. All the data were transmitted to the RESSAC staff at Cadarache.

In spring 1992, the place for the monolith extraction was prepared to allow an easy access for trucks, bulldozers and cranes. A preliminary test was also conducted to observe the stability of a large sand block surrounded by trenches, especially the hold of the angles. These observations led to some modifications of the extraction procedure of the monolith (originally described for more compact clayey soils). One week prior to excavation, the soil water table was lowered by pumping around the place of the lysimeter extraction. The excavation and sampling of the monolith were performed during the third week of August 1992. After conditioning, the lysimeter was transported by truck to Cadarache.

In the beginning of 1993, the Belgian lysimeter installed in the RESSAC greenhouse was divided into two equal parts by an stainless steel oriented North-South and sown in lines with spring wheat. In August 1993, the plants were harvested from each half lysimeters, line per line, and separated into straw and ears. Based on the number of plants compared with the amount of seeds, the mean germination was about 90% for the two half-plots ; it was somewhat higher in the Western part (93%) than in the Eastern part (87%). The production yield obtained for the whole surface indicated a good productivity (5.9 t/ha for grain and 7.2 t/ha for straw), comparable to what can be expected in the field. The production was also higher in the Western part of the

lysimeter than in the Eastern sub-plot (by 33% for straw and 7% for grain). The observation of the productivity data per line shows a higher production in the one located at the Northern border of the plot, closest to the only concrete wall of the greenhouse (see figure). Samples of the vegetation have been prepared for further analyses of their major mineral content.



Yield of straw and ear per line (90 cm) in the two halves of the Belgian lysimeter.

As the facility at Cadarache do allow to work on but one replication per soil type, it was planned to verify the reliability of the data to be obtained under controlled conditions with field data. Therefore, 12 plots (4 m^2 area) of the same sandy soil as the one sent to Cadarache have been installed at Mol. The area was fenced in order to

protect the plots from damages by rabbits. Moreover, in collaboration with the Faculty of Agronomic Sciences at Gembloux, 36 more plots were installed with the surface horizon (35 cm depth) of three different agricultural soil types, specific of surroundings of the Belgian nuclear sites (12 plots per soil type). A sample of each soil types was taken for granulometry analysis and determination of the CEC, pH and contents in carbonates, Na, K, Mg, Ca and N. All plots were sown with spring wheat.

As it will not be possible to contaminate these plots directly with radioactive dry aerosols generated by POLYR, it was decided to applied the POLYR aerosol as a wet deposit. The possibility of recovering the radioactive particles from the first pocket around the furnace is envisaged. If this procedure would appear to be valid, it will avoid to spend large amounts of money and manpower in specific shots since several pockets will be available after the shots foreseen for the contamination of the RESSAC lysimeters. A furnace pocket from a shot conducted for the TARRAS programme was brought back to the CEN/SCK for evaluating the efficiency of radioactivity recovery by washing and investigating the chemical speciation of the material washed of the terphane pocket (dialysis, electrophoresis, chromatography).

Head of Project. 4: Dr. V.R. Vallejo. Universidad de Barcelona.

The present annual progress report corresponds to the work carried out in collaboration by the Spanish participants (University of Barcelona and CIEMAT Madrid), in consequence the objectives and progress achieved are coincidents.

II. Objectives for the reporting period.

1st. Monolith sampling and installation of the lysimeter in the Cadarache-RESSAC building.
2nd. First culture implementation and its follow up.
3rd. Monitoring of phenological development of wheat plants at the lysimeter in comparison to real field conditions in Spain.
4th. Preliminary studies of root growth development.

III. Progress achieved including publications.

1st. Monolith sampling and installation of the lysimeter in the Cadarache-RESSAC building.

The Spanish monolith was extracted from the Experimental Fields of the Barcelona University, during the period 12-20/October/1992. The dimensions are 3.24m^2 of surface and 1.4m. depth, the soil type is a *Terra Rossa*. After its transport to Cadarache, the lysimeter was placed into the RESSAC building during the first week of December 1992 and the porous ground plate was installed.

2nd. First culture implementation and its follow up.

The lysimeter was divided in two equal parts in North-South direction. After the addition of the fertiliser, the surface was ploughed, levelled and sown with wheat (variety ARBON). From January to July the lysimeter was regulary watered and the usual agricultural practices were performed. In parallel the same tasks were performed in the Experimental Fields in Barcelona. The wheat was harvested in July.

3rd. Monitoring of phenological development of wheat plants at the lysimeter in comparison to real field conditions in Spain.

The control system of the climatic conditions in the greenhouse of the RESSAC building has been tested through the growth characteristics of the crops. In a first step, carried from January to July 1993, wheat has been cultivated simultaneously in the lysimeter and in the real field in Barcelona, keeping the same cultural practices. For this period, the simulated climate in the RESSAC building has been intermediate between Barcelona and Mol. Careful monitoring of phenological development of wheat plants at the lysimeter and real field has been done. At harvesting, detailed production analysis has been carried out at both sites. The main characteristics are summarized in *Table I.*

Table I.

Data from the wheat culture of the Spanish lysimeter in Cadarache. 1993

Data per m^2 (1,62 m^2 per Part).	Part A. (East).	Part B. (West).
Total weight (g).	2437,65	1553,70
Total weight of grain (g)	941,11	629,63
Total weight of straw (g)	1496,54	924,07
Number of ears	552,47	429,63
Total number of grains	15179,01	10581,48
Grains/ears	27,47	24,63

(*) including tillers with ears

Data from the parallel wheat culture in the Experimental Fields of Barcelona. (Spain). 1993.

Data per m^2 (2 m^2 per Part).	Part A. (East).	Part B. (West).
Total weight (g.).	749	535
Total weight of grain (g.).	287	207,75
Total weight of straw (g.).	462	327,25
Number of ears	329,25	276,75
Total number of grains.	7285	5275,5
Grains/ear.	22,13	19,06

Comparisons of production data obtained in the Spanish lysimeter in Cadarache and in the Experimental Fields in Barcelona is presented in *Figures 1, 2 , 3,* shown significative differences.

a) Difference between part A (East) and part B (West) in both sites. These differences are due to the delayed of time (35 days) between the first sowing in part A (20th, January) and the secod sowing in part B (24th, February).
b) Difference between the Spanish crop in the lysimeter in Cadarache and the parallel crop in the Experimental Fields in Barcelona.

The climatic greenhouse conditions in Cadarache (lower temperature and irradiation and a regular irrigation) carried during this year could be the reason of the crop vegetative stage prolongation respect to the more stressed real conditions in Barcelona. As a result of that it had been obtained a higher production in the Spanish lysimeter respect to the real crop in Spain. The production in field conditions in Barcelona (2.870 Kg. grain/Ha) was a medium-lower value for dry farming in Mediterranean zone versus the bigger production in the greenhouse conditions in Cadarache (9.411 Kg. grain/Ha).

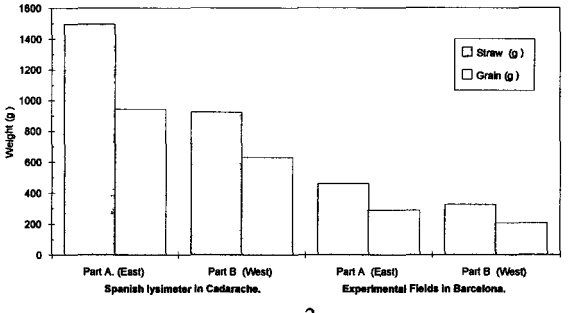


Figure 1. Crop production comparation (weight in g/m^2 of straw and grain) between the Spanish lysimeter in Cadarache and the Experimental Fields in Barcelona.

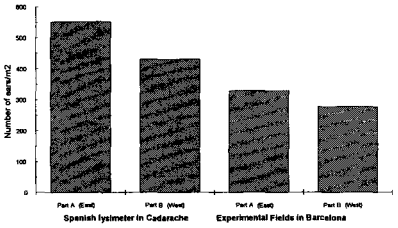


Figure 2. Number of ears/m^2 per Part (East and West) of the Spanish lysimeter in Cadarache and the Experimental Fields in Barcelona.

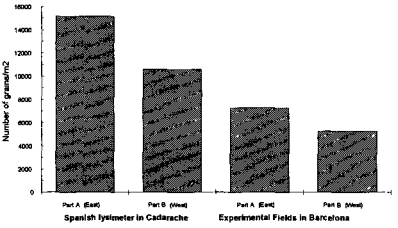


Figure 3. Number of grains/m^2 per Part (East and West) of the Spanish lysimeter in Cadarache and the Experimental Fields in Barcelona.

To check the control system of the RESSAC climate towards plant growth, a second crop of clover (*Trifolium*) has been sown in the Spanish lysimeter of the RESSAC building

4th. Preliminary studies of root growth development.

To elucidate the suitable period of time to collect the samples in the preliminary pots experiments of the next reporting period, a parallel experiment was developed using a minirhizotron system to evaluated root growth in time. These experiments were carried out in the Experimental Fields of the Facultad de Biología (Barcelona).

Objectives for the next reporting period.

1st. Detailed chemical analysis of the wheat samples.

2nd. Preliminary pots experiments using the POLYR system to evaluate the effectiveness of two countermeasures, *deep-placement* and *conventional ploughing* after the deposition of radionuclides on the soil surface, paying special attention to the role of the root distribution and it activity with respect to the radionuclides placement in the soil.

3rd. Study of differences in the soil water potential in three plots, *nude soil*, *clover* (*Trifolium*) *crop*, *mulching*. with a psychrometer system installed in the Experimental Fields of Barcelona.

4th. Study of foliar absorption and translocation of deposited radionuclides in differents stages of plant development. This study will be performed in the Spanish lysimeter in the RESSAC building in Cadarache.

5th. To compare the effectiveness of differents countermeasures, *mulching*, *ploughing* and *deep-placement* after the deposition of radionuclides on the soil surface. These experiments will be shaped according to the pots experiments results obtained previously.

Head of project 5: Dr. Förtel

II. Objectives for the reporting period

OBJECTIVES of the reporting period (september 1992 - august 1993)

The objectives of the reporting period had been based on an operational RESSAC facility in Cadarache. We aimed at dividing the monolith into three parts of equal surface and contaminating it by the POLYR device. Subsequently interception, weathering, foliar absorbtion and translocation of radionuclides from dry deposited aerosols were to be investigated.

OBJECTIVES of the following period (september 1993 - august 1994)

As the RESSAC-Programme had to bee postponed, the objectives remain the same as in the reporting period. Additionally, the substitution programme agreed upon in Cadarache on 7/8 of June 1993 will be executed.

III. Progress achieved including publications

The lysimeter has been divided by stainless steel plates beyond the plough layer. For cultivating wheat and potatoes according to good agricultural practice, german collaborators have often been present in Cadarache. We also brought fertilizers and pesticides for all lysimeters. Our scheme for fertilization and plant protection has been taken over by some european partners. Both crops were harvested on august 12/13 1993 and their fresh weight was determined.

After the harvest, clover has been sown on the whole lysimeter in order to protect the soil until the sowing for 1994 experiments. Therefore 14 g of seeds have been strewn per m^2 before slightly raking the surface.

We also made out the company, which now will deliver a prototype of the rain simulating system for the RESSAC lysimeters.

RESULTS

The results of the reporting period are neccessarily limited to the agricultural part of the RESSAC-lysimeters. The yields are presented in the tables 1 and 2. Considering that summer wheat is seldom grown in Jülich and that the Arbon variety is unknown here, the yields are in pretty well agreement with those expected.

The number of tillers and yields of grain reached in row n°1 are slightly above the mean. This is not the case for yields of straw, because tillers where shorter closed to that border. This edge effect is presumably due to light abundance on that side. In next years experiments it will be excluded by installing a fence of the type used in Jülich for that purpose.

Table 1: Yields of wheat reached on 1,08 m^2 of the German lysimeter in 1993

row	number of tillers	number of ears	weight of ears (g)		weight of straw (g)	
			fresh	dry	fresh	dry
1 (outer edge)	117	116	280.2	265.3	211.8	166.2
2	88	88	195.8	182.5	368.5	129.7
3	105	102	199.7	181.1	206.8	137.5
4	86	85	172.8	157.0	185.5	120.7
5	73	72	155.6	127.1	172.0	109.7
6	74	72	165.8	144.8	184.4	111.1
7 (inner edge)	78	77	209.8	170.7	241.5	125.0
total	621	612	1379.7	1228.5	1570.5	899.9
mean	88.7	87.4	197.1	175.5	224.4	128.6
per m^2						
total	**575.0**	**567.0**	**1277.5**	**1137.5**	**1454.1**	**833.2**
mean	**82.1**	**81.0**	**182.5**	**162.5**	**207.7**	**119.0**

The yield of grain will be available soon.

Table 2: Yields of potatoes on 1,08 m^2

plant number	fresh weight of tubers
1	920 g
2	760 g
3	650 g
4	680 g
5	940 g
6	740 g
total	**4690 g**

Head of Project. 6: J. Gutierrez. CIEMAT -Madrid-

The present annual progress report corresponds to the work carried out in collaboration by the Spanish participants (University of Barcelona and CIEMAT Madrid), in consequence the objectives and progress achieved are coincidents.

II. Objectives for the reporting period.

1st. Monolith sampling and installation of the lysimeter in the Cadarache-RESSAC building.
2nd. First culture implementation and its follow up.
3rd. Monitoring of phenological development of wheat plants at the lysimeter in comparison to real field conditions in Spain.
4th. Intercomparison exercise.

III. Progress achieved including publications.

1st. Monolith sampling and installation of the lysimeter in the Cadarache-RESSAC building.

The Spanish monolith was extracted from the Experimental Fields of the Barcelona University, during the period 12-20/October/1992. The dimensions are 3.24m^2 of surface and 1.4m. depth, the soil type is a *Terra Rossa*. After its transport to Cadarache, the lysimeter was placed into the RESSAC building during the first week of December 1992 and the porous ground plate was installed.

2nd. First culture implementation and its follow up.

The lysimeter was divided in two equal parts in North-South direction. After the addition of the fertiliser, the surface was ploughed, levelled and sown with wheat (variety ARBON). From January to July the lysimeter was regulary watered and the usual agricultural practices were performed. In parallel the same tasks were performed in the Experimental Fields in Barcelona. The wheat was harvested in July.

3rd. Monitoring of phenological development of wheat plants at the lysimeter in comparison to real field conditions in Spain.

The control system of the climatic conditions in the greenhouse of the RESSAC building has been tested through the growth characteristics of the crops. In a first step, carried from January to July 1993, wheat has been cultivated simultaneously in the lysimeter and in the real field in Barcelona, keeping the same cultural practices. For this period, the simulated climate in the RESSAC building has been intermediate between Barcelona and Mol. Careful monitoring of phenological development of wheat plants at the lysimeter and real field has been done. At harvesting, detailed production analysis has been carried out at both sites. The main characteristics are summarized in *Table I.*

Table I.

Data from the wheat culture of the Spanish lysimeter in Cadarache. 1993

Data per m^2 (1,62 m^2 per Part).	**Part A. (East).**	**Part B. (West).**
Total weight (g).	2437,65	1553,70
Total weight of grain (g.).	941,11	629,63
Total weight of straw (g.).	1496,54	924,07
Number of ears.	552,47	429,63
Total number of grains.	15179,01	10581,48
Grains/ears.	27,47	24,63

(*) including tillers with ears.

Data from the parallel wheat culture in the Experimental Fields of Barcelona. (Spain). 1993.

Data per m^2 (2 m^2 per Part).	Part A. (East).	Part B. (West).
Total weight (g.).	749	535
Total weight of grain (g.).	287	207,75
Total weight of straw (g.).	462	327,25
Number of ears.	329,25	276,75
Total number of grains.	7285	5275,5
Grains/ear.	22,13	19,06

Comparisons of production data obtained in the Spanish lysimeter in Cadarache and in the Experimental Fields in Barcelona is presented in *Figures 1, 2 , 3,* shown significative differences.

a) Difference between part A (East) and part B (West) in both sites. These differences are due to the delayed of time (35 days) between the first sowing in part A (20th, January) and the secod sowing in part B (24th, February).
b) Difference between the Spanish crop in the lysimeter in Cadarache and the parallel crop in the Experimental Fields in Barcelona.

The climatic greenhouse conditions in Cadarache (lower temperature and irradiation and a regular irrigation) carried during this year could be the reason of the crop vegetative stage prolongation respect to the more stressed real conditions in Barcelona. As a result of that it had been obtained a higher production in the Spanish lysimeter respect to the real crop in Spain. The production in field conditions in Barcelona (2.870 Kg. grain/Ha) was a medium-lower value for dry farming in Mediterranean zone versus the bigger production in the greenhouse conditions in Cadarache (9.411 Kg. grain/Ha).

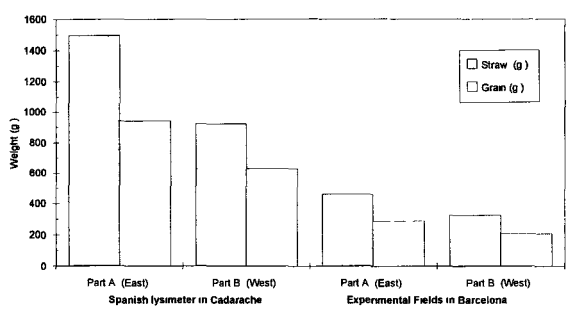


Figure 1 Crop production comparation (weight in g/m^2 of straw and grain) between the Spanish lysimeter in Cadarache and the Experimental Fields in Barcelona.

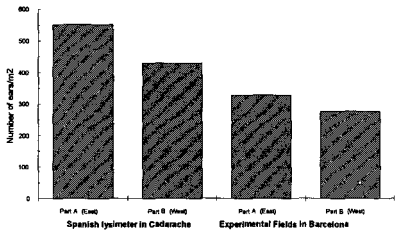


Figure 2. Number of ears/m^2 per Part (East and West) of the Spanish lysimeter in Cadarache and the Experimental Fields in Barcelona.

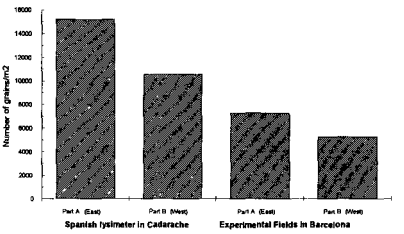


Figure 3. Number of grains/m^2 per Part (East and West) of the Spanish lysimeter in Cadarache and the Experimental Fields in Barcelona.

To check the control system of the RESSAC climate towards plant growth, a second crop of clover (*Trifolium*) has been sown in the Spanish lysimeter of the RESSAC building.

4th. Intercomparison exercise.

To ensure the validity of all the measurements that will be performed during this project, it was decided to carry out an intercomparison exercise between all the participants. The samples to measure were prepare at Cadarache three of them were artificially contamined with ^{85}Sr, ^{134}Cs (plant material, soil attack and soil) and also a sample from Chernobyl (lichen).

Head of project 7: Dr G. Arapis.

Title: Efficacity of the "non-lethal defoliation" and ecotoxicological impact of the countermeasures used for the recovery of agricultural areas.

II. Objectives for the reporting period:

The finincing of this project started only at the beginning of July 1993, and it has two main objectives:

1. To study the possibility to use the "non-lethal defoliation" to minimize the foliar uptake of radionuclides by some typical Mediterranean crops, such the vine and olive trees. This project includes also the study of radioactivity translocation, in function of the defoliation degree, to the wine and the olive-oil which are products of great economical interest in Greece.

2. To take into account the ecotoxicological impact due to the application of countermeasures involving the use of chemicals. Data on maximum tolerable doses of chemicals to be used for the recovery of agricultural lands, are expected from this project.

III. Progress achieved:

1. In-situ experiments already are made in collaboration with the CEN-Cadarache, in order to study the foliar uptake of Cs-134 (dry deposition) by the vine, grown in productive agricultural experimental field in Attiki-Greece.

2. Experimental works have started looking at of the possibility to use the "non-lethal defoliation" on real agricultural lands. Defoliation products (Harvade 60% FL and Basta 20 S.L.) are used to minimize foliar uptake and translocation of radionuclides (in function of the defoliation degree) by vine. The ecotoxicological impact of these products is analysed in order to study the productivity of the experimental agricultural area.

Head of project 8 : Ir. Kirchmann

II. Objectives for the reporting period

For the present research project, a field study on the behaviour of radioisotopes was proposed to validate the data obtained in the lysimeters of the RESSAC facilities.

In particular we planned to use 3 Belgian soil types, representative of the surroundings of Tihange and Chooz N.P.P. and for which particular transfer values were expected:

- one dystric cambisol (sol brun acide);
- one calcaric cambisol (sol brun calcaire);
- one orthic luvisol (sol brun lessivé).

From the pedological point of view, these are quite comparable, respectively, to the acidic cambisol of Flamanville (F 17), the calcaric fluvisol of Tricastin (F 2) and the orthic luvisol from Germany (KFA Jülich) that were installed in the lysimeters of the "RESSAC greenhouse" at Cadarache.

The radioecology section of the CEN/SCK has already foreseen to lead in parallel with a podzolic soil, the lysimeter experiment at Cadarache and its verification on the field plots of the CEN/SCK.

That's the reason why, after agreement with the CEN/SCK the research work is performed in close cooperation between the two institutes.

III. Progress achieved including publications

In March 1993, the superficial layers of the above mentioned 3 soil types were sampled in cultivated fields and transported to the experimental fields of the CEN/SCK Mol. There, they were placed into plots (12/soil type) surrounded by concrete plates to reach a 35 cm deep layer. This area was fenced in order to protect the plots from damages by rabbits.

The experimental plots (2 X 2 m^2) were sown by the end of April with spring wheat (var. Arbon), the same variety as the one sown on the lysimeters at Cadarache.

The 4 soil types were submitted to pedological analysis in order to verify their characteristics.

The following parameters were thus determined :

pH_{water} pH_{KCl} CEC, granulometry, exchangeable bases, $\%CaCO_3$ %N %C

The results of these analysis show that agricultural practices have little influence on the pedological parameters of the soils (CEC, exchangeable calcium).

The results obtained in the 94 experiment should validate a radioecological classification that had been adopted in the framework of a previous CEC-RESSAC contract, by gathering cartographic units according to these criteria.

Due to the impossibility to lead in 1993 the field experiment at Mol in parallel with the research in controlled conditions (greenhouses) at Cadarache, by contaminating the experimental plots with dry aerosols produced by the POLYR system, the planned experiments were postponed for one year.

It was nevertheless decided to carry out a substitution programme.

This programme aims, on one hand, to verify the similarity of the growth pattern, yield and nutritional status (macro- and micronutrients content) in spring wheat grown on different soil types in the RESSAC lysimeters and that grown on similar soil types under field conditions.

Therefore the plants will be harvested at maturity in the experimental plots of Mol, the yield will be estimated and samples will be taken for stable nutrients analysis (K, Ca, Mg, N, P, ...).

On another hand, batch experiments will be performed in order to gain more information on the expected behaviour of radiocaesium and strontium in the different soils considered in our programme. The absorption and desorption capacity will be measured in each soil type after contamination by the POLYR aerosols and by ionic forms. The availability of the radionuclides will be investigates using different methods: extraction as used in the TARRAS and CHECIR-ECP2 programmes. The change with time of these parameters will be studied.

Progress Report

Contract: **F13P-CT920049** **Sector: A26**

Title: Transfer of accidentally released radionuclides in agricultural systems.

1) Cancio CIEMAT
2) Real IPSN
3) Rauret Univ. Barcelona

I. Summary of Project Global Objetives and Achievements

The experiences following the Chernobyl accident in 1986 have emphasized the need to improve the quantification of several processes and parameters in the transfer of radionuclides along the food chain. The use of reliable parameters and accurate descriptions of processes are essential for pre-accident assessment modelling, and for the implementation of protective measures designed to mitigate the radiological consequences of an eventual accident.

The aim of this project is to contribute to the reliability of radiological assessment parameters and to establish sound scientific bases to be used in the design of post-accident countermeasures.

The global objetives of the project deal with the following aspects: i) Characterization of an accidental aerosol release containing Sr, Cs and Ag isotopes that simulate an accidental source term of PWR reactor, ii) study of the physico-chemical processes involved in their deposition on some important and common European soil-crops systems. iii) Derivation of relevant transfer factors.

In the previous periode 1990-1992 the lettuce (Lactuca sativa), a leafy vegetable, was studied. For the present phase peas (Pisum sativum), a fruit vegetable has been selected. The same soil types as in the previous phase are used: a french one (sandy soil) and a spanish one (sandy-loam soil). For the radioactive aerosol generation the french POLYR System is used and three experimental contamination are planned at Cadarache Laboratory.

The following aspects are being considered:

(a) The influence of the plant growth phase on the radionuclide retention.

Three growth stages of the plant will be contaminated in order to follow the soil-plant dynamics. Special attention will be paid to the influence of the root lenght and soil retention in the radionuclide uptake .

(b) The influence of the soil characteristics in soil migration

Soil migration and speciation in the two kinds of soils will be compared taking into account the differences in physico-chemical soil characteristics e.g. the interchange capacity of the soil, the organic matter present in them, the clay content, etc.

(c) Root uptake of radionuclides.

Root uptake by the selected crop, peas, at each plant growth stage will be followed, comparing two experiences: the first one with covered soil and the second one without the cover. Speciation studies will be done in order to quantify the radionuclides availability.

(d) Radonuclide translocation, plant interception factors and washout.

After radionuclides deposition on leaves, the plant interception factors will be analized, besides the washout effect of irrigation. Analysis of radionuclides speciation in leaves will be carried out to study the different penetration degree of isotopes.

During the reporting period two experimental contamination shoots were performed. The aerosol generated, in the first experience, was deposited on sowed peas in two soil types. The objetive has been to study the contamination level at different development stages of plants and to fruits, soil to plant transfer and radionuclide migration in soils.

The second experiment was performed on mature plants on covered soil. The aim of the experiment is to study foliar interception and translocation as a function of growth stages. Additionally, one lysimeter containing mature lettuces has been also contaminated to confirm that the behaviour of the aerosol generated with POLYR System is similar to the one previously used.

Head of Project 1: Dr. Cancio

II Objectives for the reporting period

- General coordination of the entire project
- Contribution to the experimental work
- Evaluation of the results of the aerosol transfer experiments, and determination of some parameters concerning the transfer of radionuclides in plants. Experimental data interpretation and derivation of parameters

III Objectives for the next period

- Overall coordination
- Contribution to the experimental work
- Participation in the interpretation of experimental data concerning to second and third contaminations

IV Progress achieved including publications

In this period one experiment has been carried out, two type of soil with sowed peas have been contaminated. The selected soil types, a french one (sandy) and a spanish one (sandy-loam) are the same than in the first part of the project. The culture has been changed, in this case peas plant was selected. The experiment was designed to study the soil plant transfer and radionuclide migration in soil.

Three growing stages of the plant were defined: young plant, flowering plant and fruited plant. For each plant stage soil and plant samples were taken, in the last stage, plant and fruit were sampled.

The design of the experiment, as well as the main objectives are described in the section by participant 3. In our section in this report soil migration and soil to plant transfer factor are considered.

Soil Migration

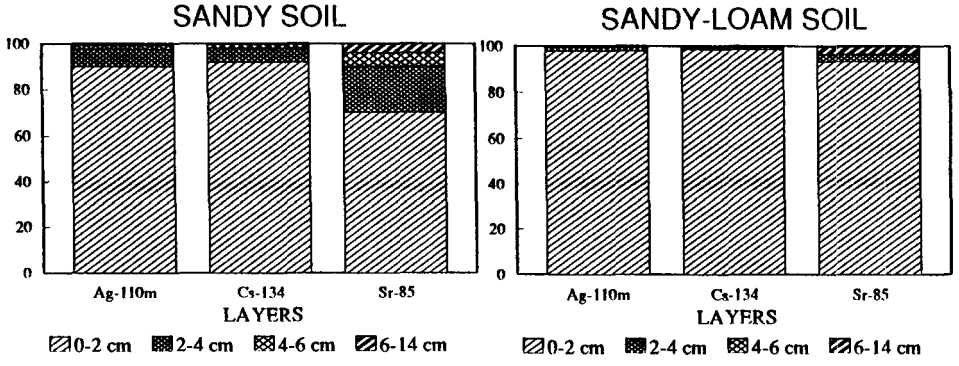
Three samples were taken at the end of experiment for total depth of soil, the samples were taken in three layers of 2 cm with depths from 0-2 cm, 2-4 cm, 4-6 cm and other from 6-14 cm (bottom of the soil).

As was expected the retention in the first layer is larger in the sandy-loam soil for the three radionuclides, the values in this case range from 98% for Ag-110m and Cs-134 to 93% for Sr-85. For the french soil the retention values range from 92% for Cs-134 to 70% for Sr-84. These results agree whith the Post-Chernobyl measurements (Graphs 1 and 2).

For both types of soil, Sr-85 is more mobile than the other radionuclides, this effect is more important for sandy soil. In general, the radionuclides remain in the layers one and two.

In this experiment with a new aerosol the behaviour of the radionuclides in soil seems to be similar than the first period (90-92). In both cases the Sr-85 is the radionuclide

most mobile, Cs-134 and Ag-110 show similar distribution for each type of soil.



Graph 1 and 2. % of the total activity in soil that remains in each layer two months after contamination

Soil to plant transfer factors

The most commonly used parameters in environmemtal modelling to describe the radiactive transport between soil and plant, is the soil to plant transfer factor TF_{sp}. It is defined as the rate of plant activity in fresh weight, to soil activity in dry weight.

The soil activity is mainly present in the two firts layers , however for TF_{sp} calculation, 14 cm deep soil samples have been taken, this soil depth is more accurate because matture plant roots reach the bottom.

Tables 1 and 2 show the plant activity in fresh weigh, and in table 4 the soil-plant transfer factor is displayed.

	Ag-110m	STD	Cs-134	STD	Sr-85	STD	% Water in plant
Young plant	27	5	13	3	607	56	90
	30	10	22	5	587	67	90
	25	6	18	4	1357	126	90
	12	5	8	3	720	66	90
Flowering plant	20	4	11	3	560	51	90
	80	12	69	8	2658	244	87
	50	10	16	4	1420	131	88
	15	9	7	2	852	75	88
Fruited plant	155	15	68	7	2022	176	86
	198	20	102	12	5106	448	86
	53	7	47	5	1883	166	86
Fruit	161	17	15	6	585	57	66
	519	48	39	8	1863	169	73
	277	27	18	6	912	84	62

Table 1. Plant Activity in Sandy-loam soil (Bq/kg(w.f))

Despite the fact of having performed 4 replicate samples. The values obtained for the four samples show high statistical deviation. This is a common feature of experiments carried out in pots and agrees with the IUR review (Handbook of Parameters Values for he Prediction of Radionuclide Transfer in Temperate Environments, IAEA Jan 1993).

	Ag-110m	STD	Cs-134	STD	Sr-85	STD	% Water in plant
Young plant	94	9	214	18	17799	1602	91
	71	11	59	9	5959	522	91
	52	10	67	10	9787	862	90
	73	8	78	7	6946	605	91
Flowering plant	74	6	64	5	11950	1042	88
	89	10	59	6	19993	1686	88
	164	19	207	21	27831	2366	86
	333	29	433	40	40787	3556	79
Fruited plant	357	38	218	23	50098	4439	79
	630	54	225	19	81516	7047	77
	181	24	107	15	67463	5742	79
Fruit	78	12	182	19	4720	414	34
	331	35	230	26	7527	666	20
	367	46	170	26	7199	623	31

Table 2. Plant activity Sandy soil (Bq/kg(w.f))

Difference with previous similar experiments with lettuce has been observed, in this case working with peas we find that the specific activity increases with the growth of plant biomass while in the experiments with lettuce the activity decreased with plant growth.

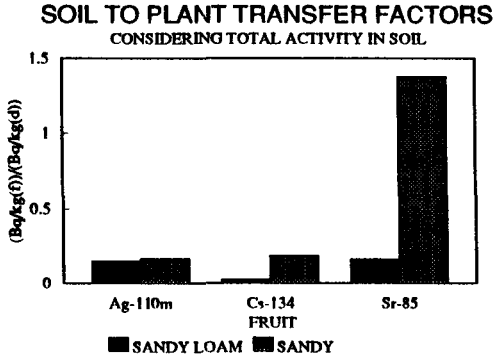
The radionuclides mobility is larger in sandy soils consecuently the transfer factors are higher due to a larger availability than other soils.

	Type of soil	Ag-110m	STD	Cs-134	STD	Sr-85	STD
Young plant	Sandy loam	0.006	0.004	0.007	0.003	0.071	0.063
	Sandy	0.034	0.021	0.080	0.138	1.773	2.724
Flowering plant	Sandy loam	0.016	0.023	0.019	0.032	0.182	0.275
	Sandy	0.089	0.128	0.163	0.242	4.854	5.873
Fruited plant	Sandy loam	0.063	0.085	0.075	0.056	0.432	0.807
	Sandy	0.248	0.449	0.173	0.167	14.094	9.961
Fruit	Sandy loam	0.149	0.209	0.025	0.026	0.161	0.294
	Sandy	0.165	0.313	0.184	0.080	1.377	0.971

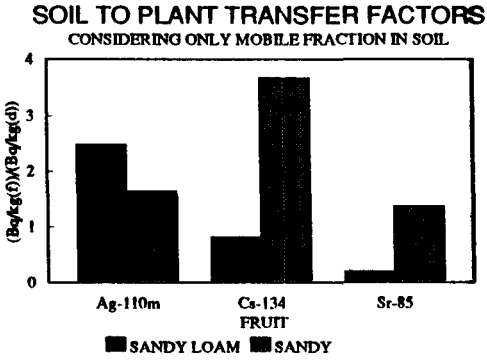
Table 3. Soil to Plant Transfer Factors (Bq/kg(w.f) plant)/(Bq/kg(w.d) soil)

From the study of the radionuclide migration in soil it can be seen that the behaviour of this new aerosol is similar to the other aerosol used in previous pariod of the project. For this reason, the soil-plant transfer factor was calculated using the mobility factors obtained in the previous study.

In the figures 3 and 4, it can be seen that the transfer factors values increase if they are calculated with the mobile radionuclide fraction in soil. The variation is much larger in the Cs-134 and Ag-110m case which mobile fraction is very small. In Sr-85 case because almost all of it is in soluble form, accessible to the plant, the variation is very small.



Graph 3



Graph 4

Head of project 2: Dra. J. Real

II Objetives for the reporting period

_ Génération et caractérisation d'aérosols radioactifs représentatifs d'un terme source accidentel, en utilisant la même installation que celle du projet RESSAC, c'est à dire le four POLYR

_ Réalisation de deux tirs permettant de contaminer des cultures de petits pois et de salades à différents stades végétatifs.

_ Poursuite des cultures dans nos serres.

III Objetives for the next period

Réalisation d'un troisième tir sur des cultures de petits pois permettant de compléter les résultats acquis lors des deux premiers.

IV Progress achieved including publications

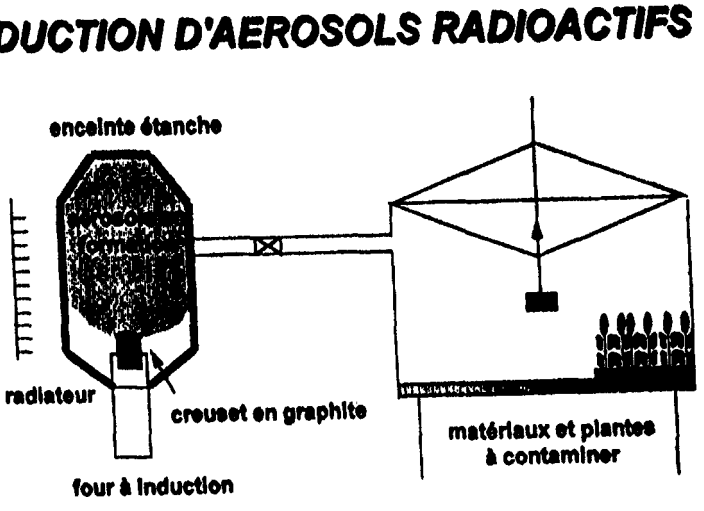
A. Caractéristiques du terme source utilisé

A.1. Caractéristiques du four POLYR

Le four POLYR est un four à induction dans lequel, un creuset de graphite contient la masse du mélange à étudier, (jusqu'à 25g). Les températures maximales dépassent les 2850°C (fusion de l'UO_2). Le creuset est surmonté d'une enveloppe chauffée à 80°C contenant de la vapeur d'eau, et simulant l'enceinte du réacteur. Les aérosols produits demeurent dans cette enceinte pendant 30 minutes avant leur transfert, ce qui permet aux réactions chimiques qui auraient lieu dans le bâtiment réacteur de se produire, notamment, la coalescence de certaines particules.

Ensuite, les aérosols sont transférés dans une deuxième poche en terphane reliée à la première par une jonction elle même en terphane comme l'indique la figure 1. Le transfert se réalise en augmentant le volume de la poche lysimètre ce qui provoque une dépression et permet d'aspirer environ 1,5m^3 provenant de l'enceinte four où se trouvent les aérosols.

Figure 1 Schéma du dispositif de contamination



A.2 Caractéristiques de la charge:

La charge est composée d'un mélange de 16 éléments représentatifs:

- du combustible, l'uranium sous la forme UO_2
- des matériaux de structure et des barres de contrôle, Fe, Zr, Cr, Ni, Ag, In, Sn, Cd.
- des produits de fission, I, Cs, Sr, Te, Ru, Ba, Ce.

Les 16 éléments sont utilisés sous la forme chimique indiquée dans le tableau 1 ci-après.

Les trois radioéléments introduits et étudiés sont le 134Cs, le 85Sr et le 110mAg. Leurs activités respectives sont données chapitre IV B.

Tableau 1. Définition de la charge type mise dans le creuset du four POLYR

ELEMENTS	Masse dans le creuset (mg)	Forme chimique dans le creuset	Masse du composé dans le creuset (mg)
Cs	173	Cs_2CO_3	212
Sr	112	$SrCO_3$	188
Te	60	Te	60
Ru	440	Ru	440
Fe	2000	Fe	2000
Cr	560	Cr	560
Ni	400	Ni	400
Ag	444	Ag	444
Cd	73	Cd	73
In	28	In	28
Sn	470	Sn	470
I	140	I	140
Zr	4200	Zr	4200
Ce	20	$Ce(NO_3),H_2O$	62

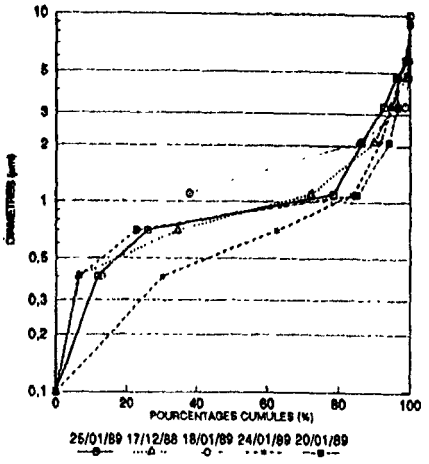
A.3 Caractéristiques des aérosols:

La caractérisation des aérosols produits a fait l'objet de nombreuses analyses et mesures.

A.3.1. Caractéristiques physiques:

Nous avons établi la courbe granulométrique des aérosols, qui donne la distribution de la taille des particules. Ces mesures ont été réalisées avec des impacteurs d'Andersen. La figure 2 ci-après représente les fréquences cumulées en pourcentage par rapport au diamètre des particules en microns, pour cinq essais. La lecture de D50 fournit le diamètre moyen de la collection de particules, nous voyons que celui-ci est compris entre 0,6 et 1,3μ. La fourchette des diamètres D25-D75 est comprise entre 0,4 et 1,8μ .

Figure 2: Courbes granulométriques



A.3.2. Caractéristiques chimiques:

Pour analyser globalement la composition chimique des aérosols, nous avons utilisé la spectrométrie de masse ICP/MS. Les aérosols soumis à analyse ont été prélevés sur les plateaux des impacteurs après pompage de l'air contenu dans l'enceinte. Le tableau 2 ci dessous donne les rendements observés de passage en aérosols sur des essais réalisés entre 1990 et 1991. Le signe / indique que l'élément n'a pu être mesuré.

Tableau 2: Composition chimique des aérosols mesurés par ICP/MS

RENDEMENTS OBSERVES DE PASSAGE EN AEROSOLS DEPOSES SUR ECHANTILLONS
MASSE SUR ECHANTILLON / MASSE DANS CREUSET

ELEM.	RAPPEL	RENDEMENTS OBTENUS en 1990 ET 1991 (%)				MOY. X	ECART TYPE	ECART TYPE σX *100
	Moyenne 88/89	02/90	03/90	06/90	01/91	(%)	σ	(%)
Fe	0,25	0,27	/	0,1	0,63	0,31	0,19	61
Zr	0,02	0,016	/	/	0,017	0,018	0,002	9,5
Cr	0,25	0,32	/	0,2	0,66	0,36	0,18	50
Ni	0,25	0,11	/	0,023	0,37	0,19	0,13	68
Ag	10	5,4	/	8,3	22,6	11,6	6,6	57
In	30	27,7	/	46,6	26,4	32,7	8,1	25
Sn	10	4,12	/	2,5	12	7,2	4	55
Cd	20	24,4	/	32,6	23,7	25,2	4,6	18
I	2	0,75	/	19,1	8,3	7,5	7,3	97
Ce	0,24	0,064	/	/	0,09	0,08	0,09	112,5
Cs	20	25	24	30	24,1	24,6	3,2	13
Sr	5	7,2	16	9,9	10,1	9,6	3,7	38,5
Te	10	21,4	/	/	24,4	18,6	6,2	33
Ru	0,016	0,012	/	/	0,09	0,04	0,04	100

ELEM. : Eléments
MOY. : Moyenne

La détermination des espèces chimiques a été faite par diffraction X, méthode ne permettant de distinguer que les phases cristallines. Ces mesures ont permis en particulier d'identifier Ag métal non oxydé, Sr métal qui dans l'air se transforme rapidement en Sr(OH)2, Cs métal se transformant aussi rapidement en hydroxyde de césium.

B. Conditions expérimentales

Deux tirs ont été réalisés, en utilisant comme radioéléments intégrés dans la charge définie précédemment, le 134 césium, le 85 strontium et le 110m argent.

Les activités de départ dans la charge étaient de $3,5.10^7$ Bq pour le césium, $1,8.10^8$ Bq pour le strontium et $3,5.10^7$ Bq pour l'argent.

Lors de la contamination nous installons dans l'enceinte qui contient les végétaux, des filtres en acétate de cellulose, également répartis sur la surface. Après mesure de ces filtres par spectrométrie γ nous pouvons vérifier l'activité déposée ainsi que son homogénéité dans l'enceinte.

Le tableau 4 donne les caractéristiques radioactives des filtres pour les deux tirs concernés.

Tableau 4 Caractéristiques des filtres

	Tir du 04/11/1992			Tir du 17/05/1993		
	85 Sr	110m Ag	134 Cs	85 Sr	110m Ag	134 Cs
moyenne	1,47.10+6	5,55.10+5	3,43.10+5	4,23.10+5	1,95.10+5	9,79.10+4
écart-type	1,31.10+5	4,41.10+4	3,46.10+4	1,10.10+5	5,12.10+4	2,41.10+4
valeur max.	1,74.10+6	6,59.10+5	4,27.10+5	7,72.10+5	3,57.10+5	1,67.10+5
valeur min.	1,19.10+6	4,59.10+5	2,73.10+5	2,66.10+5	1,19.10+5	5,97.10+4

La suite des résultats expérimentaux est explicitée par les partenaires 1 et 3.

Head of project 3: Dra. G. Rauret

II. Objectives for the reporting period.

Objectives 1st. shoot:

- Radionuclides migration in soil profile.
- Contamination level at different development stages and fruits.
- Root uptake.

III. Objectives for the next period.

Completation and interpretation of the 2nd. shoot results:

- Total interception.
- Washout due to simulated rain.
- Leaf speciations before washout and at the end of experience.
- Direct absorption.
- Comparison between two types of aerosol used in TARRAS project.
- Contamination level of the fruit.
- Radionuclides aging in soil (speciation).

Objectives 3th. shoot:

- Total interception.
- Direct foliar absorption.
- Retranslocation at diferent development etages.
- Root uptake.
- Contamination level at diferent development etages.

IV. Progress achived including publications.

A.) Experimental design.

A.1) Lisymeters preparation.

Soil. Two types of soil has been used (like during the first period of TARRAS project), Soil 1: from Barcelona, sandy-loam and Soil 2: from Belleville, sandy.

The soil was 1 cm sieved. For each lisymeter, the same amount of soil has been weigh and put in.

Fertilization. Before to sow, the fertilizer (N:P:K 30:80:120) has been mixed homogenicaly into the soil: 5g per lysimeter.

Irrigation and drainage. Manual sprinkling 3 times a week (monday, wednesday, friday) noting the quantity and the date in a table. The quantity of water has been dependent of necessity (variable with climatic conditions) and the same amount of water has been used for each lisymeter containing the same type of soil.

For covered soil, the irrigation has been doing drop by drop (directly in the soil) and manual sprinkling for plants washout. The throughfall water has been collected and analysed too.

Lisymeter was supplied with false bottom in order to assure free drainage.

The samples must be taken from a fixed area (like in shoot 1).

In order to undestant the water washout effect, some lisymeters with drop by drop irrigation was analized too.

Plants.

- For the 1st. shoot:

* sowing density ..... 2 lines x 12 seed x 1 lisymeter

(the lisymeter that will be used for take samples at young plant stage, was more density sowed, in order to avoid enough plant material for analisis)

* plants number at mature stage ..... 2 lines x 5 plants x 1 lisymeter

- For the others shoots:

At contamination time, the plant mass was aproximately equal for the diferent lisymeters containing one soil type.

A.2) Sampling

Four replicates of each sample (for plant and for soils) has been taken out.

1st. shoot .....october 1992

2 m^2	sandy-loam soil (soil 1)	sowed peas
2 m^2	sandy soil (soil 2)	sowed peas

Next table is the same for soil 1 and soil 2.

One lisymeter was reserved for to study the evolution (migration and aging) of radionuclides during a long period (aprox. 1 year).

Sampling timetable

DEVELOPMENT STAGE	SOIL SAMPLES	PLANT SAMPLES
sowed soil (contamination time)	25 cm^2x 2 cm depth * 1 sample for soil speciation	-
young pea plants	For a fixed area: * 0-2 cm depth * 2 cm to the bottom	1/2 of lisymeter area: * aerial part * roots
flowering	For a fixed area: * 0-2 cm depth * 2 cm to the bottom	1/2 of lisymeter area: * aerial part * roots
fructification	For a fixed area: * 0-2 cm depth * 2-4 cm " * 4-6 cm " * 6 cm to the bottom	1/2 of lisymeter area: * fruits * aerial part * roots

2on shoot .....march-april 1993
1 m^2 mature lettuces
2 m^2 fructified pea plants, covered soil

The plants has been cultivated on soil 2 (from Belleville).
The soil was covered to prevent the soil contamination, that can gave the opportunity to root absorption.

Sampling timetable.

WASHOUT	LETTUCE	PEA PLANTS
After contamination (no washout)	* aerial part * leaf extractions	* fruits * aerial part * leaf and pod extractions
1st washout	* water washout	* water washout
2on washout	* aerial part * water washout	* fruits * aerial part * water washout
3th washout	* water washout	* water washout
4th washout	* aerial part * leaf extractions * water washout	* fruits * aerial part * leaf and pod extractions * water washout

A.3) Sequential extraction scheme applied in vegetation samples.
A plant was weighed, sequentially extracted and its activity measured. The extraction procedure applied were as follows:
STEP 1: The plant was manually immersed and gently shaken in 500 ml distilled water for 1 minute. The particulate fraction was then separated from the soluble one by filtration through a 0.45 μm filter. Both, filter and liquid activities were measured.
STEP 2: The plant, previously extracted with water, was manually immersed and gently shaken in 500 ml of chloroform. As in step 1, the particulate fraction was separated from the soluble one by filtration through a 0.45 μm filter. Both, filter, liquid as well as residual activities were measured.

B.) Results.

B) Radionuclides migration in soil profile.

The results are refered to shoot 1.

B.1) Migration over time.

Figure 1 shows the radionuclides migration over time in both soils. Sampling soil were performed at the same time of the plant sampling (S:seedling, Y:young plant and M:mature plant). It can be observed that Sr migration is always higher than Cs and Ag migration, according to the lower Sr soil fixation. All radionuclides show a decrease in the activity found in the first soil layer (0-2 cm).

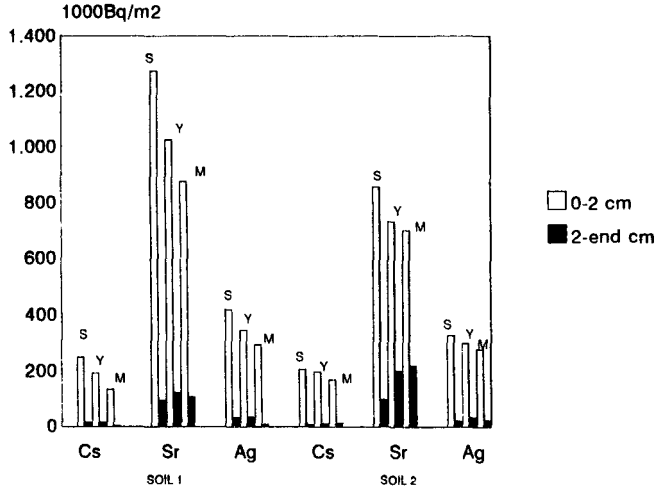


Figure 1. Soil radionuclide migration over time (S:seedling, Y:young plant and M:mature plant).

B.2) Soil depth radionuclides distribution at the end of experiment.

Table 1 shows the mean value of three replicates of radionuclide activity (Bq) in each soil profile layers two months after contamination. The radionuclide migration depends on the type soil. In soil 2 the migration is higher than in soil 1.

Table 1. Mean value of radionuclide activity (Bq) in each soil profile layers.

	^{110m}Ag	^{134}Cs	^{85}Sr
SOIL 1			
Depth: 0-2 cm	4998 ± 2438	2287 ± 1367	14815 ± 6214
2-4 cm	96 ± 63	30 ± 15	577 ± 448
4-6 cm	14 ± 7	4 ± 2	50 ± 28
6-14 cm	43 ± 56	17 ± 25	1205 ± 1104
SOIL 2			
Depth: 0-2 cm	4043 ± 1166	2788 ± 948	8968 ± 2466
2-4 cm	312 ± 254	183 ± 176	2270 ± 1342
4-6 cm	57 ± 35	35 ± 21	576 ± 189
6-14 cm	106 ± 81	37 ± 17	1793 ± 830

B.3) Contamination level at different development stages and fruits.

Figure 2 shows that the specific activity in plant increase amount growing period for both soils except for Cs in soil 2. In fruits Cs and Sr specific activity are lower than in the rest of the aerial plant. Ag seems to be accumulated in fruits.

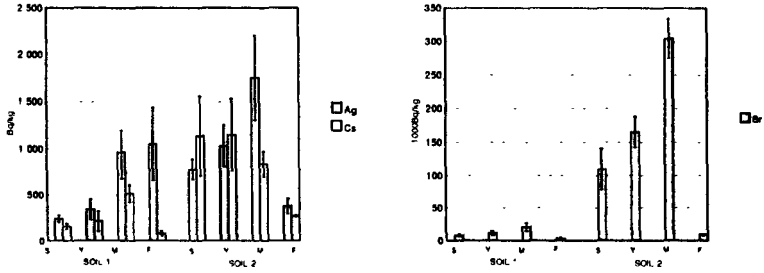


Figure 2. Specific activity in plant at different growth stages.(S:seedling, Y:young plant, M:mature plant).

B.4) Root uptake.

Figure 3 shows the total root surface in each plant growth stage. In soil 1 the total root surface increase during growing period whereas in soil 2, a decrease at the last plant sampling was observed. This different behaviour observed for both soils could be due to more accelerated development in plants growing in soil 2 and, at the last sampling, the plants in soil 2 were more mature than in soil 1. Total root surface over time has the same behaviour than Cs specific activity in plants (Figure 3).

Figure 4 shows root depth distribution at the end of the experiment (mature plants). The root surface increase with depth whereas the specific activity in soil layers has the inverse distribution, but this low activity in the lower soil layers seems to be enought for plant absorption and consequently, plant contamination.

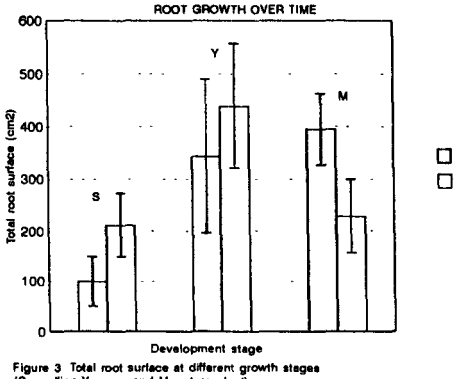


Figure 3 Total root surface at different growth stages (S seedling,Y young and M mature plant)

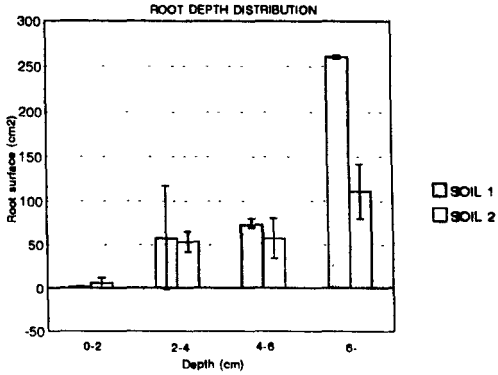


Figure 4 Root depth distribution in mature plant

Publications

1) M.Vidal and G. Rauret;"Two approaches for sequential extraction of radionuclides in soils:batch and column methods". *Inter.J.Environ.Anal.Chem.*,Vol.51,pp.85-95,1993.

2) J.Tent, M.Vidal, M.Llauradó, G.Rauret, J.Real and P.Mischler;"Sequential extraction as a tool to study foliar uptake of radionuclides". *J.Radioanalytical and Nuclear Chem.*,Vol.173,No.2,1993.

3) M.Vidal and G.Rauret;"Prediction capacity of a Sequential Extraction Scheme". *J.Radioanalytical and Nuclear Chem.*, (pendiente de publicación)B:

Progress Report

Contract: FI3P-CT930071 **Sector: A26**

Title: Influence of the food-processing techniques on the level of radionuclides in foodstuffs.

1)	Colle	CEA - Cadarache
2)	Cawse	UKAEA
3)	Grandison	Univ. Reading

I. Summary of Project Global Objectives and Achievements

An initial collaborative research program between the participants has been made since 1990, during two years within the CEC "TARRAS" project. It has demonstrated that food processing can either decrease or increase the radionuclide concentrations in the final products.
More detailed examination of basic processes is now required to explore the ways to reduce radionuclide concentrations in foodstuffs.

Different collaborative actions have started concerning:
- the influence of different processing technologies and of additives on the radionuclide content of vegetables,
- and, the fate of some radionuclides that enter factory by-products intended for animal feed.

A bibliographical work was started to review specific papers and statistics concerning recycled by-products from different industrial processes. Industrial samples will be analysed for cesium, strontium and potassium.

The main part of the programm concerns the influence of some parameters used in canning processes of vegetables on removal of radionuclides. This part is made by using vegetables cultivated in some field plots established near Sellafield Works or contaminated artificially.

The elements which are studied are K, Cs, Sr, Ru, Ag and Co.

Parameters to be tested are:
- additives,
- time of storage,
- crops varieties,
- processing technologies

The experimentations are now in progress.

Head of project 1: Dr. Colle

II Objectives for the reporting period

Le but du programme est d'améliorer les connaissances relatives au comportement des radioéléments au cours des processus de transformation alimentaire, notamment en ce qui concerne:
- l'effet des traitements technologiques sur la teneur finale en radioéléments des conserves de légumes,
- l'étude de certains sous-produits des industries alimentaires utilisés pour la fabrication des aliments pour animaux.

III Progress achieved including publications

Les travaux de la période écoulée ont porté sur les points suivants:
- recherche bibliographique et prise de contact avec les industriels.
- mise au point et démarrage d'essais expérimentaux de conserves de légumes

Résultats des recherches bibliographiques et contacts avec les industriels.

Les sujets abordés n'ont pas fait l'objet de nombreux travaux, en particulier pour ce qui concerne les transferts de radioéléments aux aliments industriels pour animaux. Toutefois, on a rassemblé des données quantitatives concernant la part des sous-produits dans les aliments pour animaux.
En ce qui concerne les additifs et auxilliaires de fabrication, une revue des textes législatifs communautaires a permis de choisir parmi les produits utilisables en conserverie de légumes ceux dont l'étude est envisagée.

Expérimentations

Expériences préliminaires: comparaison des résultats selon le mode d'obtention des végétaux

L'étude en laboratoire de conserves effectuées à partir de légumes cultivés sur un sol contaminé artificiellement s'avère problématique car d'importantes quantités de sol et de radioéléments sont nécessaires pour obtenir une activité suffisante dans les végétaux bruts, avec de plus les éventuels aléas liés aux cultures en zone contaminée. Nous avons effectué les cultures sur vermiculite et solution nutritive, sous éclairage artificiel. La contamination est effectuée à mâturité des légumes par trempage durant 48 h soit des racines (haricot vert), soit des feuilles (carottes) dans une solution contaminée, immédiatement avant la mise en conserve.

Lors d'une première expérimentation nous avons effectué une mise en conserve à partir de haricots et de carottes contaminés par ces deux techniques. Les végétaux ont subi un parage (prélavage, épluchage, lavage), un blanchiement (5mn) à l'eau, puis une stérilisation (30mn) dans de l'eau + NaCl 2% . Après 1 mois, les conserves ont été égouttées et rincées. Le tableau 1. montre que les résultats des transferts aux légumes en conserve sont comparables.

Tableau 1. : Comparaison des facteurs de transfert (Bq/kg produit final/Bq/kg produit initial) obtenus selon le mode de contamination des végétaux:
a) culture sur sol enrichi en radioélément avant semis
b) contamination à mâturité par une solution radioactive

	^{57}Co		^{134}Cs		^{85}Sr	
	a	b	a	b	a	b
Haricot vert entier -> blanchi	0,85	0,85	0,79	0,74	0,86	0,67
Haricot vert entier -> conserve	0,31	0,37	0,24	0,31	0,46	0,37
Carotte parée -> conserve	0,16	0,14	0,18	0,15		
Carotte entière -> conserve	0,06	0,08	0,06	0,05		

Etude expérimentale de l'effet de divers paramètres

Sur des carottes contaminées à maturité par trempage des feuilles durant 48 h dans une solution enrichie en ^{57}Co, ^{106}Ru et ^{134}Cs, on a effectué, après parage (prélavage, épluchage, lavage), divers traitements (tableau 2.).

Le parage élimine de 70 à 80 % de la radioactivité initiale (essentiellement lors de l'épluchage), ensuite, le blanchiment à l'eau se démarque des autres traitements en améliorant l'efficacité de la décontamination à partir du parage par rapport aux autres traitements.

Tableau 2. : Effet du type de traitement après parage. Expérimentation sur carottes contaminées à maturité.

Processus après parage			Activité résiduelle (% de la carotte parée).		
blanchiment	stérilisation (30mn): jus	délai de stock-age	^{57}Co	^{106}Ru	^{134}Cs
eau 5mn	eau + NaCl 2%	1 mois	17	< 12	21
vapeur 5mn	eau + NaCl 2%	1 mois	32	49	28
vapeur 5mn + cuisson 30mn	eau + NaCl 0,3%	1 mois	30	53	25
-	eau + NaCl 2%	1 mois	28	(60)	23
-	eau + NaCl 2%	2 mois	29	(53)	24

Remarquons que le délai avant l'ouverture des boites n'a pas d'effet significatif, comme on peut également l'observer tableau 3. .

Tableau 3. : Effet du délai de stockage avant ouverture des boites sur l'activité résiduelle du haricot en conserve (en % du haricot brut).

délai (mois)	1	2	3	4
^{57}Co	31	30	37	32
^{134}Cs	24	15	26	23
^{85}Sr	50	42	49	44

Enfin, les essais concenant les additifs sont en cours: des expériences sur l'effet d'un jutage avec ou sans NaCl ont été réalisés sur le haricot vert pour le Sr, Cs, Co, Ru. L'ajout de sel à 2% dans le jus permet d'augmenter la décontamination du haricot vert en conserve de 10% pour le strontium.
Des expérimentations analogues sont en cours pour la carotte et en prévision pour la tomate. L'influence d'un pelage chimique doit également être étudié. Dans ce but, des cultures sont faites en permanence sur milieu artificiel afin de disposer de végétaux prêts à être contaminés et traités.

Head of project 2: Dr. Cawse

II. Objectives for the reporting period

The objectives for the period January to August 1993 were:

(i) to cultivate food products from a region of Great Britain having relatively high concentrations of Sr-90 and Cs-137 in soil, for processing at Reading University Department of Food Science and Technology,

(ii) to prepare raw and processed foods food for analysis of K-40, Sr-90, Cs-137 and their stable elements,

(iii) to cultivate and/or obtain mature leafy vegetables for contamination by soluble radiotracers of Ru-106 and Ag-110m,

(iv) to seek information on the fate of waste material that is produced by processing of a variety of food products by different methods, in order to derive a semi-quantitative inventory of the annual loss of food solids and hence potential Sr-90 and Cs-137 content at given levels of contamination (see Project 3).

III. Progress achieved including publications

A. The influence of processing methods and additives on radionuclide concentrations in foods

The Sellafield region of Cumbria, North West England, was chosen for cultivation by Harwell of carrots, potatoes and peas. This region has relatively high concentrations of Sr-90 and Cs-137 in soil owing to historical releases of radionuclides from Sellafield Works and fallout from the Chernobyl accident in 1986. Environmental surveys by Harwell have shown concentrations of Sr-90 are ~ 10 Bq/kg dry soil, and ~ 60 Bq/kg for Cs-137.

Varieties of carrots, potatoes and peas were selected according to their use by the food processing industry in Great Britain. The carrot variety 'Narman', the potato variety 'Maris Peer' and pea variety 'Bunting' were chosen because of their wide use in canning processes.

Carrots, potatoes and peas were sown during April 1993 at a long established field plot on farmland close to Sellafield Works. Pesticides were applied to the crops at regular intervals to optimise yields. All crops were harvested during July and August 1993 and their yields recorded.

Raw and processed foods have recently been produced using facilities at Reading University Department of Food and Science and Technology. Details of processing methods used for the vegetables are described in Project 3, by Reading University. Samples of the raw and processed foods are now undergoing preparation for analysis of K-40, Sr-90, Cs-137 and their stable elements.

B. The influence of food processing on removal of radionuclides deposited to foliage of mature leafy vegetables

Spinach and oilseed rape crops are currently being cultivated under greenhouse conditions at Harwell. These crops were sown in May 1993 and it is anticipated that they will mature in Autumn 1993. Mature crops of cabbage and cauliflower are being sought also. All crops are to be contaminated by the application of soluble radiotracers of Ru-106 and Ag-110m. Losses of radioactivity will be recorded after processing, by normal culinary methods in the case of cabbage, cauliflower and spinach; solvent extraction methods used in the production of rape seed oil are being investigated.

C. The fate of radionuclides in by-products and wastes from food processing

Information is being sought on the Fate of "waste" material that is produced by the processing of potatoes, oil seed, wheat, milk and sugar beet, using different methods. Samples of raw and "by-product" material of the foods are being obtained by Reading University (see Project 3), for analysis of stable Sr and Cs; thus, a semi-quantitative inventory of the annual loss of food solids and hence potential Sr-90 and Cs-137 content at given levels of contamination is to be derived.

Head of project 3: Dr. Grandison

II Objectives for the reporting period

Objectives for the period January to August 1993 were:

i) To process materials having relatively high concentrations of Sr-90 and Cs-137 using different processing methods, for analysis of K-40, Sr-90 and Cs-137 and their stable elements at AEA/Harwell (see Project 2).

ii) To seek information on the fate of waste material from the processing of a variety of food products in order to derive a semi-quantitative inventory of the annual loss of food solids and hence potential Sr-90 and Cs-137 content at given levels of contamination. The study concentrates on products which are used for animal feed.

III Progress achieved including publications

i) The influence of processing methods and additives on radionuclide concentrations in food

Varieties of carrots peas and potatoes were selected according to their use by the UK food processing industry for canning as follows:

Carrots	Narman
Peas	Bunting
Potatoes	Maris Peer

Raw materials containing relatively high levels of radionuclides were produced as described in Project 3.

Canning is carried out using standard processing methods but altering the composition of blanching waters and canning brines. Up to 2% KCl has been incorporated into these solutions in an attempt to produce a substantial reduction in the levels of some radionuclides.

The pea processing has been completed and samples delivered to AEA/Harwell for analysis after 1 month's storage. Processing of potatoes and carrots is in progress.

ii) The fate of radionuclides in by-products and wastes from processing

A survey of government statistics and other published material on the production and utilisation of potatoes, rape seed, wheat and sugar beet has been carried out, with particular reference to wastes and by-products which are incorporated into animal-feed. Also meetings have taken place with personnel from companies involved with processing these commodities.

Samples of raw materials, products and by-products from the processing of rape seed, sugar beet and wheat have been obtained for analysis of Sr and Cs at Harwell.

Work is continuing on assembling statistics from the dairy industry and obtaining appropriate samples for analysis.

A semi-quantitative inventory of potential losses of Sr-90 and Cs-137 into these by-products and wastes, and hence into animal feeds, will thus be carried out.

III B

FOLGEN DER STRAHLENEXPOSITION DES MENSCHEN; IHRE ABSCHÄTZUNG, VERHÜNTUNG UND BEHANDLUNG

CONSEQUENCES OF RADIATION EXPOSURE TO MAN; THEIR ASSESSMENT, PREVENTION AND TREATMENT.

CONSEQUENCES POUR L'HOMME DE L'EXPOSITION AUX RAYONNEMENTS, EVALUATION, PREVENTION ET TRAITEMENT

Progress Report

Contract: **F13P-CT920030** **Sector: B1A**

Title: Co-operative research on late somatic effects of ionizing radiation in the mammalian organism.

1) Maisin EULEP

I. Summary of Project Global Objectives and Achievements

The objective of the European Late Effects Project Group (EULEP) is to improve the understanding of late biological effects of exposure to ionising radiation. Its work consists of the standardization and development of methodology in the member institutions, the co-ordination and promotion of co-operative research by means of task groups, and the organisation of training activities, workshops and symposia. There are now 25 laboratories participating in this work.

Work on the Standardisation and Development of Methodology has been carried out by the following committees:

- Committee of External Radiation Dosimetry and Techniques
- Committee of Internal Radiation Dosimetry and Techniques
- Committee on Pathology
- Committee of Cell and Molecular Biology
- Expert Group on Physiological Methodology

The co-ordination of collaborative research work between the member institutions has been organised by means of a number of problem-orientated task groups and special task group actions. A related objective has been the establishment of an archive of long-term radiobiological studies.

EULEP is concerned to promote the training of young radiobiologists. High priority continues to be attached to the support of scientific exchange visits between laboratories for the purpose of acquiring technical expertise. The means by which EULEP achieves its stated objectives is reviewed regularly, and it has maintained sufficient flexibility in its organisation to meet changing requirements.

Head of Project 1: Prof Maisin

II. Objectives for the reporting period

(a) to continue to develop the programme of standardisation and development of methodology through the committees;

(b) to promote the co-ordination of research by the task groups and special task group actions, including the review of existing work and the establishment of new task groups and actions as opportunities presented themselves; to continue development of the radiobiology archive;

(c) to prepare a detailed submission to the Commission on priorities for late effects research for the period 1985-1999.

Objectives for the Next Reporting Period

These will be broadly as under (a) and (b) above. In addition EULEP will complete a degree of reorganisation, which has been in preparation during the last year. This will enable EULEP to co-ordinate its activities better in relation to the overall research programme of the Commission.

III. Progress achieved including publications

STANDARDISATION AND DEVELOPMENT OF METHODOLOGY

Committee of Pathology and Committee of Cell and Molecular Biology

These two Committees have worked in close conjunction, especially in the organisation of a workshop on the pathology of transgenic mice on the occasion of the General Assembly at Reisensburg. Several experts gave talks covering many aspects of the application of transgenic mice, especially the study of the pathogenesis of malignant lymphoma and mammary neoplasia. Not only EULEP members but also pathologists from outside participated in this discussion.

The annual slide seminar was held on 6 November at Neuherberg on the "Pathology of Immune-Related Diseases". Professor Müller-Hermelink (Würzburg) gave an excellent overview on the pathogenesis of immunopathic diseases. He demonstrated that the morphologically defined diseases, e.g. Hashimoto's thyroiditis, Lupus arteriitis, myasthenia gravis, MALT, and gastritis might rely on different initiating stimuli. Professor Baroni demonstrated the role of expression of cell adhesion molecules and cytokines in human thymus and thymomas. Professor Feller (Lübeck) gave an overview of the interactions in the cytokine network during the maturation of lymphoid cells and the role of the disturbance of this network in the pathogenesis of malignant lymphoma. He demonstrated examples of IL-2 knockout mice with a lesion similar to human ulcerative colitis. The role of autocrine stimulation of lymphoid cells by IL-9 was illustrated by cases of large cell malignant lymphoma in IL-9 transgenic mice and implants of IL-9 transfected murine T cells. In addition he showed the corresponding lesions in humans. Dr Feichtinger (Innsbruck) and

Professor Boniver (Liège) demonstrated their experimental models of AIDS. Boniver's model is the murine AIDS ("MAIDS") i.e. progressive lymphoproliferative lymph node lesions (lymphadenopathy) after infection of 4-5 week old C57BL mice with RadLV-Duplan. Feichtinger's model is the cynomolgus macaque after infection with SIV. He followed the viral infection with molecular biological methods. In his slides he showed a peculiar giant cell disease of lymph nodes and lung. In addition, problem cases of liver pathology in rats from Professor Bannasch (Heidelberg) were discussed.

The revised form of the chapters of the first volume of the new edition of the EULEP Atlas (now printed by Schattauer Verlag) is nearly complete.

Committee of External Radiation Dosimetry

The last EULEP X-ray dosimetry intercomparison has once again stressed the need for all participating institutes to adhere to the EULEP protocol for X-ray dosimetry and exposure arrangements (Zoetelief *et al*, 1985, EULEP Protocol for X-ray dosimetry, CEC report EUR 9507).

The activities of the committee have now been focused on the characteristics of phantoms simulating experimental animals of various sizes. In this context the EULEP phantoms have also been included in the ICRU report 48 (1993) on Phantoms and Computational Models in Therapy, Diagnosis and Protection. As a follow-up of the studies with the rectangular and cylindrical rat phantoms, dose distributions have been studied in more realistic phantoms. For unilateral and bilateral irradiation with 300 kV X-rays of the real shape phantoms, maximum to minimum doses values in the trunk amounted to 2.5 and 1.25 respectively. These values are appreciably higher than those reported earlier (Zoetelief *et al*, 1985, Int. J. Radiat. Biol. 47, 81-102) for phantoms with simple geometry.

The present activities of the EULEP Dosimetry Committee are concerned with the assessment of dose distributions employing Monte Carlo calculations and the introduction of new thermo-luminescent material for the determination of the absorbed dose.

Committee of Internal Radiation Dosimetry

The Committee met during the General Assembly in March 1993 and reviewed the role of certain task groups (see below). It became clear that, even where some task groups would close, EULEP would be able, by means of new groupings, to maintain a useful and productive interest in areas such as the evaluation of new chelating agents and the dosimetry of internal emitters in bone.

The Committee continued to support standardisation activities by means of inter-laboratory consultations.

Many of its members had been involved with ICRP and other bodies developing biokinetic models for age-dependent dosimetry, the new lung model, and the guidebook for dealing with contamination of workers by radionuclides.

For the future, the Committee aims to involve itself more in training activities, especially in computer-based dosimetry.

Expert Group on Physiological Methodology

Regarding late effects after whole body irradiation for bone marrow transplantation, the results presented by the European Bone Marrow Transplantation Group represent an epidemiological study related to the frequency of some non-specific functional disturbances occurring after different conditioning procedures. It is felt that before considering a relationship between whole body irradiation and delayed clinically-observed symptoms, a conditioning procedure protocol, accepted by every participant, is urgently required. The validity of the subsequent observations is highly dependent on this condition. Moreover, the success of such a study depends also on the agreement of the participating centres on a common protocol defining criteria for the selected physiopathological investigations as well as on a procedure for their quality control. Only those functions in directly related systems or organs at highest risk should be considered. Highly sensitive, highly specific and easy-to-perform techniques have to be available.

With this in mind, the follow-up of the hormonal status related to the thalamo-hypophyseal tract, to thyroid and gonadal functions, as tested by radioimmunoassays or any other means using small serum samples, are good examples of what could be done, particularly on young patients. Another example is cataract detection by visual examination. Particular attention is also drawn to the respiratory system. The alveolar-capillary wall is a high-risk target and the measurement of lung diffusion capacity for CO is a highly sensitive way of detecting changes in the transfer capacity from air to blood in the lungs.

As far as the victims of the Chernobyl accident are concerned, a similar protocol could be proposed, with the possibility of an interesting comparison between controlled and uncontrolled whole body irradiation.

CO-ORDINATION OF COLLABORATIVE RESEARCH

The future of EULEP task groups has been the subject of an in-depth review. It was generally recognised that part or all of the co-ordination activities of some task groups had in one sense been superseded by the establishment of multi-national contracts. Accordingly some task groups have been disbanded, having completed and published their joint work - e.g., the groups on reduction of risk, fetal dosimetry, stem cells and bone. In the cases of bone and reduction of risk, new groups have been established (see below).

Selected reports on progress achieved by some of the task groups are as follows:

Interspecies Comparison of Lung Clearance

The next interspecies of lung clearance using physically and chemically uniform terbium oxide particles is under way, in which 7 laboratories will participate including human volunteers and 4 animal species: monkeys, dogs, HMT rats, Fischer-344 rats, Sprague-Dawley rats and CBA/H mice. The first 2 batches of uniform terbium oxide test particles have been prepared and neutron-activated. They were used for pilot lung clearance studies in HMT rats after inhalation and instillation, and also for metabolic studies. The results of these studies will be used to select the appropriate particle properties for the interspecies comparison of lung clearance which is planned to start in 1994.

Another interspecies comparison of lung clearance using physically and chemically uniform uranium oxide particles is in preparation. Two lines are envisaged: (1) a long-term (≥ 6 months) interspecies comparison on rodents and dogs or monkeys using $^{233}U_3O_8$ particles; (2) a short-term (3 weeks) interspecies comparison including human volunteers using $^{236}U_3O_8$ labelled with ^{237}U by neutron activation.

In addition to measurements of the intracellular particle dissolution (IPD) in cultured alveolar macrophages (AM) of dogs and F-344 rats using monodisperse porous $^{57}Co_3O_4$ particles, there are now IPD data available from AM of humans, baboons, HMT rats and CBA/H mice. All these species had participated in the first two interspecies comparisons using uniform $^{57}Co_3O_4$ particles. The results confirm that AM of all species are capable of dissolving these test particles significantly faster than extracellular body fluids. They confirm in part that IPD in AM *in vivo* caused the differences in absorption of dissolved particle material from the lungs to blood found previously in the various species. In order to standardize the technique of IPD measurements in the two laboratories using this method (MRC & GSF), parallel studies have been carried on AM obtained from baboons which had undergone broncho-alveolar lavage at CEA Bruyères-le-Châtel. First results showed similar kinetics of IPD when the AM were transported appropriately between the laboratories.

Meetings to plan the above interlaboratory studies were held at Erfurt on 4 October 1992 and at Reisensburg on 8-9 March 1993.

Deposition and Clearance of Inhaled Particles in the Human Respiratory Tract

The objective of this joint EULEP-EURADOS task group is to promote and facilitate collaboration between European laboratories involved in the development of improved respiratory tract models for intakes of radionuclides. Its approach is to identify important areas of uncertainty, and to conduct experiments to address them. (Several members were also members of the ICRP task group on Respiratory Tract Models.)

Experimental studies were carried out to quantify aspects of human respiratory tract deposition and clearance. Deposition measurements focused on areas where data are sparse, e.g., partition of air between nose and mouth, total deposition in children.

Clearance studies continued to focus on particle retention in the bronchial tree, because of the high radiosensitivity attributed to the bronchial epithelium. The most recent human studies indicate that retention is related to particle size, and this was taken into account in the new, recently adopted ICRP model. The first stages of a standardisation exercise, planned at the task group meetings, were carried out successfully. The initial objective is to investigate differences in results reported by KI Stockholm and GSF Frankfurt for deposition and bronchial clearance of particles. A joint experiment has also been planned to examine the effects of broncho-constriction on bolus deposition and clearance. The human studies are complemented by experiments on rats and dogs, in which the site of deposition is more readily specified.

Measurements also continued in a three-year study of the retention of particles in the alveolar region of the human lung.

Meetings to plan the above studies were held at Erfurt on 5-6 October 1992 and at Reisensburg on 10 March 1993.

Reduction of Risk of Late Effects from Incorporated Radionuclides

The year has been notable for the considerable progress achieved with the siderophore analogue 3,4,3-LIHOPO. The substance was synthesised by Professor Burgada and colleagues at Univ. Pierre et Marie Curie, Paris with the collaboration of Professor Raymond, Univ. of California. Collaborative animal studies at Karlsruhe, Bruyères-le-Châtel and NRPB have shown that after different modes of intake of Pu and Am, 3,4,3-LIHOPO is considerably more effective than the current agent of choice, DTPA. Preliminary studies have shown that the ligand is also more effective for Th. Detailed studies on the toxicological symptoms of 3,4,3-LIHOPO in the baboon, involving histological examination of liver and kidney and biochemical analyses of blood and urine, are being undertaken at Fontenay-aux-Roses. So far the results are most encouraging.

Studies undertaken on the administration of DTPA in drinking water at NRPB and Karlsruhe have shown that this can be an effective method of treatment for Pu and Am but further work is required to optimise the regimen. Work on the testing of calixarene derivatives form removing uranium from the body has been undertaken at Pierrelatte. New diphosphonate derivatives synthesised at the Univ. Pierre et Marie Curie have also been examined at Pierrelatte and NRPB. Whilst some of the substances have been partially effective, the development of more effective ligands remains a priority.

New chelating agents for removing Pu from the body including wound sites have been investigated by Dr Rencova (Prague) in collaboration with Karlsruhe and Vanderbilt University, Tennessee. The most promising substances identified so far are derivatives of demethyldithiocarbamate.

It was agreed that the EULEP collaboration should in future be concerned with the evaluation of new chelators for the purpose of identifying project areas or new ligands worthy of further investigation. Meetings to review progress and discuss future work were held at Pierrelatte on 21-22 October 1992 and at Reisensburg, March 1993.

Effects of Radon and Radon Daughters

The aims of the radon task group for this year were to complete the intercomparisons of radon metrology in the European animal radon exposure facilities and to establish methodology for the determination of deposition of radon daughters in the respiratory tract. The second intercomparison of radon metrology was conducted at the CEA facility at Razes in June 1992. The third intercomparison was held at the TNO facility in Rijswijk in May 1993. Scientists from CEA, TNO and AEA participated in these studies. At each laboratory, the following measurements were made: potential alpha energy concentration (PAEC), individual radon daughter levels, "unattached" radon daughter fractions and $^{214}Bi/^{214}Pb$ deposited in animals. Prior to these measurements, the participating laboratories exchanged their counting standards and measured each others' sampling flow rates. The participants agreed to within 10% for both flow and activity measurements. Comparison of measurements made of radon daughters showed a greater variation, in the case of PAEC 25%. These differences must be due to differences in sampling geometry and timing. The

deposition of $^{214}Bi/^{214}Pb$ in the lungs, trachea and head of rats exposed for at least three hours was estimated using gamma spectrometry. The results showed inconsistent variations between participants, even though all three made measurements on the same animals. The reasons for the variations were discussed at 'Indoor Radon Remedial Action', a CEC conference held in Rimini, June 1993. The results of the first two intercomparisons were presented at this meeting. The studies conducted to date have highlighted considerable variations in measurements of radon daughter deposition. The reasons appear to be due to different methods for decay correction. It has therefore been decided that the next intercomparison exercise to be conducted at AEA Harwell will investigate the reasons for the discrepancies with each group, using the counting data from other groups as well as the same animals to estimate the deposition of radon daughters.

The task group held a meeting at Erfurt in October 1992, to discuss the results of the intercomparison, and actions to remedy the problems which had been highlighted. A further task group meeting was held at Reisensburg, March 1993.

Standardisation of Bone and Bone Marrow Dosimetry

At the General Assembly held at Reisensburg in March 1993, members of the former bone task group discussed work including ongoing long-term studies of animal tumorigenesis. A common interest identified was the lack of information on the distribution of dose within bone and bone marrow in relation to observed effects. Similarly, work with *in vitro* bone cultures had not involved supporting work on distribution of dose. These discussions lead to the suggestion that a new task group should be formed with the specific objective of developing approaches to the estimation of dose distribution within bone and bone marrow. The following aims were therefore agreed:

1. standardisation of methods for the calculation of doses to the whole skeleton, the periosteal and endosteal bone surfaces, bone volume and bone marrow;

2. correlation of the results obtained with findings from studies of incorporated α-emitters;

3. the "*in vitro*" cell dosimetry of α-emitters.

The first full meeting would be held later in the year.

Developmental Effects

A new task group was established in 1992 combining co-operative programmes on pre-implantation mouse embryos and on developmental effects on the CNS. This group can be expected to improve research co-ordination, in view of the overlap of many aspects in these two fields of study.

At a meeting at Harwell on 26-27 October 1992, recent research was reviewed and future priorities for co-ordination of research were defined in the following areas:

A. Developmental effects induced during oogenesis or during the pre-implantation stage

- risk of malformation after X-ray exposure of the various stages of oogenesis at low dose rates;
- effects of radiation on female germ cells of the guinea pig and on the pre-implantation mouse embryo.

B. Developmental effects induced during neurogenesis

- cellular (neuronal) damage studied *in vitro* and *in vivo*;
- neuro-structural damage and growth retardation: effects in rat brain after chronic pre-natal exposure at low doses, and cortical radiation damage at different dose rates;
- neurofunctional disorders: behavioural effects of pre-natal exposure on spatial memory function in adult mice.

It was agreed that until now radiation research on embryonic effects had been mainly directed towards the quantification of specific effects, related to dose and modifying factors. The mechanisms inherent in the induction and transfer of developmental damage had been neglected. Future studies would aim to improve insights into "response chains" leading to the different end-points at the various levels of biological organisation from the genome to the whole organ.

Radiation Effects in the Urinary Bladder

The common basis of the ongoing co-operative study are experiments on radiation-induced changes in bladder compliance, which have been completed in all participating laboratories. The results of these functional studies form the basis for comparative morphological and pathogenetic studies. In order to rule out experimental factors which could possibly hamper an interlaboratory comparison of such results, factors modulating urinary bladder compliance in mice were studied. Sequential morphological studies covering a time period of 2-360 days after bladder irradiation with the ED80 and the ED20 respectively are ongoing: in Amsterdam and in Munich all animals have been irradiated and most of them have already been sacrificed and bladders embedded in paraffin and glycolmethacrylate. Pilot studies have shown that immunohistochemical detection of collagen type I and III is feasible in our material; however, the staining protocols are currently being optimised. Detection of TGFβ was first established in human skin scar material, but then proved to be difficult in mouse tissues. Currently a different antibody is being tested. Radiation-induced changes in bladder epithelium cell kinetics are being studied in continuous BUdR labelling *in vivo*.

EULEP External Symposium

A symposium entitled "Acute and Late Effects on the Lungs from Inhaled Radioactive Materials and External Radiation" was held on 4 October 1992, during the 24th Annual Meeting of the European Society for Radiation Biology at Erfurt, Germany. There were eight invited presentations covering the field of acute and late effects caused by inhaled radioactive materials as well as by external radiation of the lung. The first part covered aspects of the new respiratory tract dosimetry model proposed by the ICRP task group; there were related presentations on particle deposition and clearance and on cells at risk. The second part of the symposium was devoted to radon, including recent animal experiments as

well as epidemiological investigations. Abstracts of the symposium were published in the EULEP Newsletter 73, May 1993.

Joint BETG/EULEP Workshop on Lung Pathology

A workshop was held in Paris on 12-13 October 1992, organised jointly by the Biological Effects task group of the US Department of Energy and by EULEP. The primary objective of the workshop was to compare, and as far as possible to standardise, the nomenclature and classification of lung tumours and they arise in the rat following exposure to ionising radiation. A secondary aim was to review future research priorities for radiation carcinogenesis investigated by animal experimentation with associated pathological studies. A report was submitted both to the DOE and to the CEC, published later in the EULEP Newsletter 73, May 1993.

Publications

EULEP Newsletter: 5 issues were published during the reporting period (Nos. 69-73).

EULEP Submission to the CEC (1993): *Research in Radiobiology as Applied to Radiation Protection - Proposals for the Period 1995-1999.*

European Radiobiological Archive of Animal Experiments (ERA), Part I, List of Communicated Experiments, eds. G.B. Gerber and W. Gössner.

Report of Joint BETG/EULEP Workshop on Lung Pathology (1993), resulting from a workshop held on 12-13 October 1992 in Paris.

Progress Report

Contract: FI3P-CT920027 Sector: B11

Title: Biophysical models for the effectiveness of different radiations.

1)	Paretzke	GSF
2)	Goodhead	MRC
3)	Terrissol	ADPA
4)	Leenhouts	RIVM
5)	Von Sonntag	Inst. Max-Planck
6)	Smith	Univ. London - Physics
7)	O'Neill	MRC

I. Summary of Project Global Objectives and Achievements

In this proposal, experimental, computational, and system analytical work is foreseen to improve the present knowledge on the shape of the dose-time-effect curves especially in the low dose regime of radiation protection and its dependence on the time dependence of dose delivery (rate and fractionation), the type of radiation (X, gamma, alpha, neutrons and mixed fields) and on the influence of environmental factors.

The scientific approach to develop such an integral mechanistic (i.e. not empirical) model for radiation carcinogenesis is structured in this project into the development of several separate, serial models for the different levels of biological complexity, namely for:

a) the induction by radiation action of primary and secondary physical and early chemical changes in the DNA (by Terrisol et al., O'Neill et al., v. Sonntag et al.),

b) the subsequent formation of mutations (e.g. HPRT$^-$) and chromosome aberrations (Paretzke et al., Goodhead et al.),

c) the induction of cellular changes and late somatic effects in experimental animals and man by such early cell effects including intercomparison with the mechanistic action of UV-light (Leenhouts et al., Paretzke et al.).

Particular emphasis will be given to the mechanistic interpretation of the HPRT-mutation and the induction of lung tumors by alpha emitters.

In all areas of this experimental and theoretical project significant progress could be achieved which is reported in detail in the following chapters.

Head of project 1: Dr Paretzke

II. Objectives for the reporting period

There were three areas of research objectives during this reporting period :

(i) Physico-chemical models :
to extend the cross section basis used in track structure calculations to protons and to condensed targets an experiment was performed to measure secondary electron spectra produced by MeV protons in thin carbon foils (in cooperation with the Radiological Physics Laboratory, Battelle Pacific Northwest Laboratories, Richland, USA);
in addition, calculation were performed with the existing track structure codes to intercompare results with other partners in this coordinated project;

(ii) Biological, cellular model :
to improve the simulation of realistic cellular targets in consideration of the extensive computer memory requirements of three dimensional heterogeneous voxel descriptions of a mammalian cell a feasibility study was performed to use Marching-Cubes Algorithms for the storage of the surfaces only of cellular regions;

(iii) Multi-step cancer model :
to provide the epidemiological basis for the intercomparison of mechanistic models for the induction of lung tumors in members of the public by the inhalation of Rn-daughter products several empirical models for this endpoint (BEIR IV, modified BEIR V, Jacobi model) were compared in a probability of causation plot.

III. Progress achieved including publications

(i) Physico-chemical models : In an ultra-high vacuum chamber the angular and energy differential spectra of electrons ejected from a 5 $\mu g/cm^2$ Carbon foil by 1 and 2 MeV proton impact (pulsed beam) were measured with a time of flight detector in the energy range between 1 and 100 eV (which comprises about 95% of the ejected electrons). First, for calibration purposes, the electrons ejected by protons from Xe-gas were compared to theoretical and other experimental data; the good agreement found suggested the proper functioning of the apparatus. However, the spectra measured for the condensed target showed a strong time dependency (see Fig. 1) indicating the influence of an residual gas adsorption layer forming on the Carbon surface with increasing time. This effect affected even relatively high electron energies (up to 100 eV) which is unexpected and complicates evaluation of the results in terms of primary emission cross sections. To overcome this problem a chamber is in construction which permits even lower residual gas pressures.

For the intercomparison of results obtained by different approaches used by the different authors of this coordinated project to describe relevant properties of charged particle tracks extensive calculations were performed with the GSF-code PARTRAC which were reported at the 11th Symposium on Microdosimetry, Gatlinburg, 1992. The main problems realized were the present ignorance of decay modes of biological molecules in the realistic liquid phase in a cellular environment.

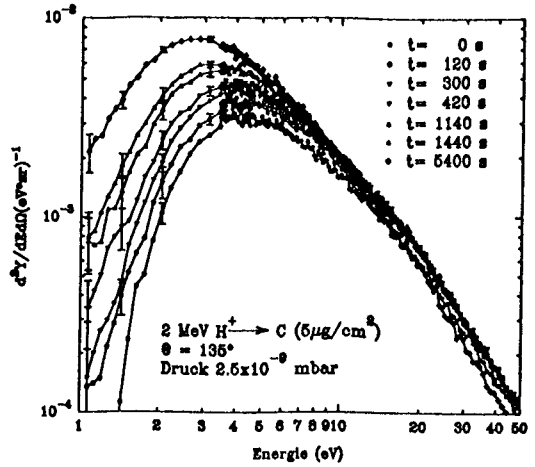


Fig. 1 : Absolute double differential electron spectra emitted at 135 ° from a thin carbon foil by 2 MeV protons.

(ii) Biological-cellular model : The simulation in a computer of a realistic cell (with DNA structure) is a complicated problem requiring compromises between spatial resolution on one hand and storage and computer time requests on the other hand. To improve this situation the feasability of various algorithms for the description of surfaces instead of three dimensional volumes was tested. In particluar the Cuberille-Model for the generation of polygonal surfaces and the Marching - Cubes Algorithm creating triangle models of surfaces using classification of cube types and look-up tables was investigated (see Fig. 2). The preliminary results are indicating that in this way lower storage and speed requirements might be needed in track structure simulations.

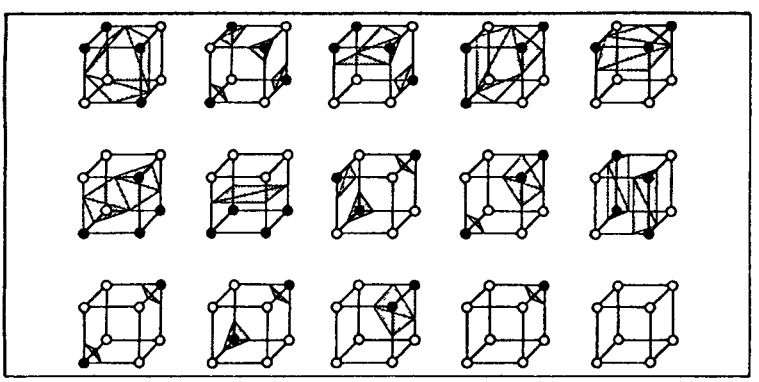


Fig. 2 : The 15 basic situations of voxels in the Marching Cubes Algorithm.

(iii) Multi-step cancer model: Before mechanistic models for lung cancer induction by ionizing radiation can be developed and compared to the epidemiological data it was useful to study the different existing empirical models for this endpoint which were proposed in the literature to describe the lung cancer incidence among the survivors of the atomic bomb explosions in Hiroshima and Nagasaki as well as for the underground Uranium

miners. Fig. 3 shows the plot of the excess relative risk surface over the age at exposure and age at expression of risk plane according to the model of Jacobi for the WISMUT underground miners after an exposure of 100 WLM. Significant differences were found between this model and that of the BEIR IV committee for the same occupational exposure and that of the BEIR V committee for the external short term irradiation with low LET radiation at Hiroshima and Nagasaki. This finding will complicate the comparison of an mechanistic model at the high dose range were epidemiological data exist.

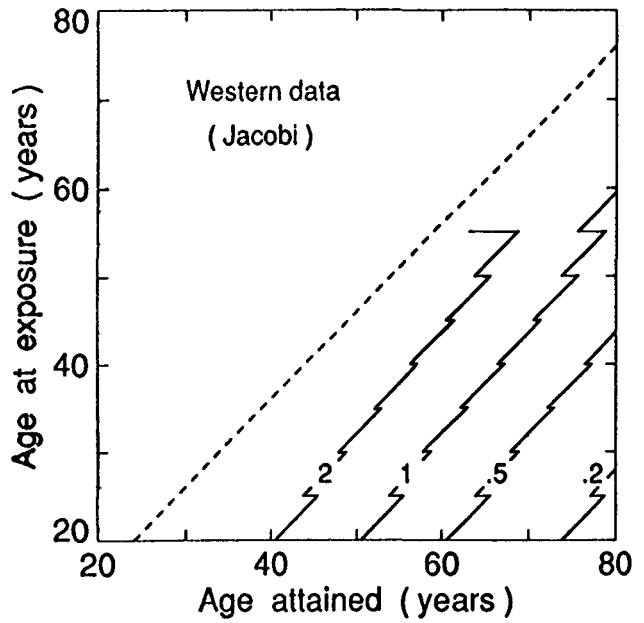


Fig. 3 : Predictions of the Jacobi model for the excess relative risk after 100 WLM as a function of age at exposure and at risk.

The two publications resulting from this work are given in Project 2 (MRC-Phys) and 3 (ADPA).

Head of project 2: Dr. Goodhead

II. Objectives for the reporting period

1. Assess robustness of scoring results, at the nanometre level, by comparisons using Monte Carlo transport codes from different workers.
2. Extend electron transport code for electrons from 100 keV up to the MeV region.
3. Initiate compilation of available information on *hprt* mutagenesis and supplement with new experiments where appropriate.
4. Accumulate data on track structure, DNA damage and single-track survival probabilities required for understanding and modelling mutagenesis.

Objectives for next reporting period

Develop methods for incorporating main chemical pathways in track structure modelling of damage to DNA. Further comparison of track codes. Assistance in development of a generalized code format.

Complete α-particle dose rate *hprt* mutation experiments. Complete modifications for hypoxic irradiations and commence *hprt* mutation experiments. Compile α-particle survival factors for diverse cell types and evaluate effects on observed mutation frequencies. Continue with MRC(Chem) on α-particle and ultrasoft X-ray induced DNA damage measurements and modification of chemical environment, to guide development and testing of track structure modelling.

III. Progress achieved, including publications

Progress on the related components of the contracts was as follows:

(a) Track structure calculations

We have previously established an extensive data base on absolute frequencies of energy deposition in cylindrical volumes of dimensions < 1 to > 100 nm for a wide variety of different radiations, simulated by MOCA8b and MOCA14 Monte Carlo codes, to act as a guide towards the biologically critical features of radiation quality and the nature and quantities of relevant initial molecular damage in cells. Calculations also included preliminary evaluations of DNA strand breakage from direct ionizations in the DNA. As more advanced evaluations are approached, including more refined descriptions of the DNA and inclusion of the chemical pathways, it becomes particularly important to judge the reliability of the track simulations at very high spatial resolution. For such purposes we have obtained statistically representative numbers of electron tracks of selected initial energies from four other water transport codes world wide, in addition to MOCA8b. These have been compared through our large-statistics scoring routines on the basis of the resultant absolute frequencies of energy deposition in cylinders of diameters 2 and 25 nm [1]. In many cases agreement was good but substantial differences appeared over dimensions of a few nanometres, especially for larger energy depositions. These differences were not entirely eliminated even when relative frequencies for different electron energies were considered.

In collaboration with S. Uehara, electron interaction cross sections have now been extended from 100 keV (the limit of MOCA8b) up to several MeV to allow simulation of these higher energy electrons. The new code KURBEC has been fully described [2].

Work has commenced on formulating scoring procedures for DNA damage both from track interactions occurring directly in the DNA and from those that produce water radiolysis products using the chemical-track code of Terrissol, with a view to assessing the frequencies and complexities of clustered damage, including strand breaks and altered bases, and comparing these with experiment information from other partners in the contract.

(b) Experimental studies of radiation quality in cellular systems

Compilation has commenced on pertinent data in the literature on *hprt* radiation-mutagenesis, with emphasis on studies revealing dependence on radiation quality, dose and time [3]. This mutation system has been selected as a particularly suitable prototype for mechanistic understanding and modelling because of the extensive information that already exists on radiation induction and molecular characterization. Direct experiments are being undertaken to fill some of the notable gaps in available information. These include the effects of reduced dose rate for high-LET radiations and dependence on oxygenation for both low- and high-LET radiations. Initial experiments with 121 keV μm^{-1} α-particles showed a clearly significant increase in mutation frequency in plateau phase V79 fibroblasts when irradiated over 40 h rather than a few minutes. However, ongoing subsequent experiments are showing that this occurs only when titanium-(as opposed to glass)-walled dishes are used, so the titanium observation is of peripheral interest. Comparative data for chromosome aberration induction and cell inactivation (in glass) have been obtained. A new system is being designed for our α-particle irradiator to allow multiple-dish (up to 20) irradiations of cells with controlled gassing atmospheres for hypoxic irradiations to modify the DNA damage.

Mutations of the *hprt* gene are also being measured in other cell types, namely in a variety of developmental stages of haemopoietic B cells (in collaboration with S. Griffiths and M. Greaves) and in peripheral T lymphocytes (with M. Thompson and B. Bridges), with X-rays and α-particles to ascertain the generality of the induced frequencies and the radiation-quality dependence. An essential feature for interpretation and modelling of these results is assessment of the probability S_1 that a cell will survive the passage of a single α-particle and therefore remain capable of expressing genetic damage. Our results with murine marrow haemopoietic stem cells [4] have shown that for these S_1 is quite small (10-20% for 121 keV μm^{-1} α-particles) compared to the much larger values for fibroblasts (typically 60->90% from the literature). For such evaluations, we now routinely include in our experiments confocal microscope measurements of the dimensions of the living cells, and their nuclei, under the conditions of irradiation. We are also evaluating S_1 factors for radiosensitive fibroblasts, such as AT human cells and *irs* hamster mutants, as potential factors that may be partially responsible for their apparently reduced dependence of cellular effects on radiation quality.

Our experimental work with α-particles has included collaborations with partner MRC(Chem) on a variety of fronts to establish the yield, nature and repairability [5] of the initial DNA damage, with special emphasis on the clustered damage [6] and other features that may be revealed by the track structure analyses of (a) above. Conditions are being established for some corresponding experiments with ultrasoft X-rays, as a means of enriching for the clustered contribution from the low-energy electrons that occur in all low-LET irradiations. These low energy electrons have been postulated as being the biologically dominant component of all low LET radiations but the evidence to date is by no means conclusive [7].

(c) Track features related to mutagenic effectiveness

In order to understand and quantify radiation-induced mutagenesis, track features must be considered at both the DNA and cellular levels [8]. Clusterings of initial ionizations at the DNA level or slightly larger (5-10 nm) have been suggested as important features in determining the radiation quality dependence [9, 10]. For the production of DNA double-strand breaks (as a bulk class) there is apparently little dependence on radiation quality, possibly because fairly small clusters may be dominant in producing dsb [11]. Most final cellular effects show considerably greater dependence on radiation quality, possibly reflecting the lower repairability of larger clusters. Current data suggests that this may be particularly so for mutation.

(d) Publications

[1] H. Nikjoo, M. Terrissol, R.N. Hamm, J.E. Turner, S. Uehara, H.G. Paretzke and D.T. Goodhead. "Comparison of energy deposition in small cylindrical volumes by electrons generated by Monte Carlo track structure codes for gaseous and liquid water". Radiat. Prot. Dosim. (in press, 1993).

[2] S. Uehara, H. Nikjoo and D.T. Goodhead. "Cross sections for water vapour for Monte Carlo electron track structure code from 10 eV to MeV region". Phys. Med. Biol. (in press, 1993).

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Head of project 3: Dr. Terrissol

II. Objectives for the reporting period

During this contract period our objective was to simulate the more precisely possible the physical and chemical steps of the irradiation of the DNA contained in the cell nucleus. We wanted to include a more realistic description of the DNA structure for the primary interactions of radiation and then to take into account all chemical reactions between water radio-chemical species and radicals with sub molecular units of DNA.

III. Progress achieved including publications

Until now, all biophysical models used to do statistical investigations, derived interactions on DNA from interactions inside cylinders of water. To be nearer the real target, we decided to introduce DNA, employing the three spatial co-ordinates of all the single atoms of the double helix molecule.

Our first basic model is a staight segment of DNA included in liquid water, the co-ordinates are taken from X-ray diffraction studies. Since direct interaction cross-sections for electron on DNA are not yet well known, we used the van der Waals radius of the DNA atoms. The electrons slow down in liquid water containing the DNA segment : during the physical step (up to 10^{-15} s.) all events located inside the van der Waals radius of a DNA atom are scored as direct interactions on DNA while other water interactions create species and radicals. As time increases, from 10^{-15} s. to 10^{-6} s., all water species e^-_{aq}, $OH^\cdot$, $H^\cdot$, H_3O^+, H_2O_2, OH^-, H_2 , HO_2 diffuse and react between themselves (26 possible reactions), as well as with DNA sub-units. For the latter reactions we adapted experimental data (Table-1) taken from the literature.

Table-1: Reactions radii in nanometers of main water species with DNA sub-units, calculated from reaction rate constants.

	OH	e-aq	H
Deoxyribose-monophosphate	0.085	0.0003	0.0006
Adenine	0.288	0.265	0.0019
Cytosine	0.288	0.382	0.017
Guanine	0.425	0.413	0.000
Thymine	0.302	0.53	0.011
Formate ion	0.142	0.000	0.004
Tris	0.071	0.000	0.000

The rates were measured at room temperature, not in exact conditions of a cell nucleus, but it is a first attempt. At present we assume too, that these rates do not vary with local gradient of species or radicals concentration.

It will be now possible with the introduction of scavengers to distinguish between direct and indirect effects. As an example, we expect that every ionisation on phosphate or sugar atoms creates a direct single strand break; an indirect is assumed when a deoxyribose monophosphate reacts with a water radio product. A first version of the code will be used with partners 2 (MRC-Phys), 5 (MRC-Chem) and 7 (MPI) to evaluate parameters needed, check the code and test the different assumptions.

H. NIKJOO, M. TERRISSOL : " Monte Carlo track structure studies of the chemical modification of initial damage in DNA". Conference on Pathways to Radiation Damage in DNA, Oakland University, Rochester, Michigan , June 1992.

D.T. GOODHEAD, H.P. LEENHOUTS, H.G. PARETZKE, M. TERRISSOL : "Track structure approaches to the interpretation of radiation effects in DNA". Eleventh Symposium on Microdosimetry, Gatlinburg, Tennessee, September 1992. To be publish in Radiation Protection Dosimetry (1993).

E. POMPLUN, M. TERRISSOL : "Contribution of direct and indirect effects to strand break induction by DNA bound 125 Iodine" 24th Meeting of the European Society for Radiation Biology, Erfurt, Germany, Octobre 1992.

H. NIKJOO, M. TERRISSOL , R.N. HAMM, J.E. TURNER, S. UEHARA, H.G. PARETZKE, D.T. GOODHEAD : " Comparison of energy deposition in small cylindrical volumes by electrons generated by Monte Carlo track structure codes for gaseous and liquid water" Eleventh Symposium on Microdosimetry, Gatlinburg, Tennessee, September 1992. To be publish in Radiation Protection Dosimetry (1993).

M. ROCH : "Simulation des effets des physiques et chimiques des rayonnements ionisants au niveau de l'ADN". Doctorat de l'Université Paul Sabatier n° 1327, Toulouse, November 1992.

M. TERRISSOL, E. POMPLUN : "Computer simulation of DNA incorporated 125 Iodine Auger cascades and of the associated radiation chemistry in aqueous solution" Eleventh Symposium on Microdosimetry, Gatlinburg, Tennessee, September 1992. To be publish in Radiation Protection Dosimetry (1993).

Head of project 4: Dr Leenhouts

II. Objectives of the reporting period

a) Track structure model: application of the track structure model to the analysis of the initial slope of the dose-effect relationship of radiobiological data and the comparison of the results with modelling results of the other participants in the contract.
b) Stationary CHO cells: cell culture experiments will be used to compare the dose effect relationship of cytotoxicity for gamma rays and UV, and the possible interaction of both agents. In a later stage the interaction will also be investigated for the induction of a mutation.
c) Application of a two-mutation model for carcinogenesis to the analysis of experimental data on tumour induction in animals.

III. Progress achieved including publications

a) Track structure:
The track structure model (TRAX) is now available for the application to experimental data. At the 10th Microdosimetry Symposium (1992) results of this model were presented in a common paper of 4 participants of this contract in an attempt to compare the different approaches for describing the effectiveness of different radiation types.

b) Stationary CHO cells:
In this period the experiments of exposure of stationary CHO cells to gamma rays and UV for cytotoxicity were extended with introducing the HPRT mutation as another cellular endpoint. The aim of another endpoint is to compare the results of combined exposure to gamma rays and UV for cytotoxicity with results for mutation induction and investigate the ratio for lethal and mutagenic effect under different conditions.
The standard procedure for scoring HPRT mutations was adapted for applicability to the CHO cell system. Roughly, cells which were plated, subcultured and allowed to grow for 10 days after the irradiation treatment, were tested for resistance against 5 μg/mL thioguanine in the next 7 days and from the colonies still formed the originally mutated cells were calculated. Experimental results were obtained for both 300 nm ultraviolet radiation (UV) and for gamma rays. In fig 1 results of the dose-effect relationship of HPRT-mutations for exposure to 300 nm UV are shown. The dose response is supra-linear and can be described by a purely quadratic function of the exposure. These resuluts are in agreement with the assumption that the mutations are caused by pairs of pyrimidine dimers on opposite strands of the DNA.

Experiments are planned to investigate the mutations after combined exposure to UV and gamma rays to investigate the possible synergistic action of the agents.

c) Analysis of radiation carcinogenesis:
The first attempts to analyse experimental data using the two-mutation model of carcinogenesis were continued. Firstly, the combined model of the two-mutation carcinogenesis model and the cellular radiation biology model was standardised for general applicability. For example, parameters were introduced to define a lag time t_0 for the (minimum) period between formation of the malignant cell and appearance of the first tumour, and an expression time t_1 for the total period over which tumours which come from

mutagenic rates of both mutational steps are assumed to be equal) and will be used in the model as long as experimental data do not require a more detailed approach.

Qualitatively, the combined model has some unique characteristics:
a) the model describes the development of tumours starting from molecular damage at DNA-level up to the detection of the tumour in the animal (or man), and takes into account the biology of the carcinogenesis process.
b) the model simultaneously accounts for the development of tumours with age of the animal and for the radiation dependence. This implies that the model describes the interaction of spontaneous and radiation induced tumours.
c) the model has characteristics attached to the absolute and the relative risk projection model; in general, for exposure of young animals the risk projection with age resembles the relative risk projection model, while for exposure of older animals the risk projection is according to the absolute model (see fig. 1).

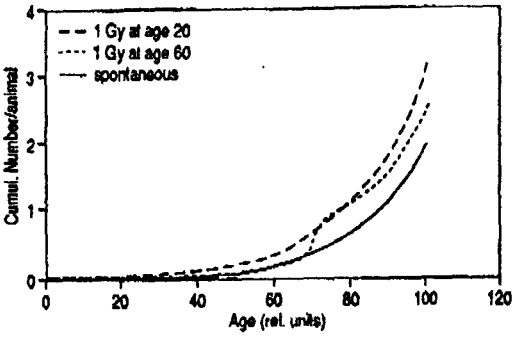


Fig. 1 Cumulative tumours with age in a hypothetical animal arising spontaneously, and after a short exposure at age of 20 and 60, showing an increase proportional with the spontaneous tumours (relative lifetime projection) and as a constant number (absolute lifetime projection), respectively.

d) the mathematics of the model shows that, in cases where animals have a relatively high spontaneous tumour incidence, the dose-effect relationship resembles a single-mutation response, while for animals with low spontaneous tumour incidence the dose-effect relationship resembles a double-mutation response.
e) if the model is proven to be generally applicable the model allows for extrapolation of data to tumour incidence under different exposure regimes. For example, using experimental or epidemiological data for short term exposures the model can predict the effect of lifetime exposures. And using data from animals or populations with low spontaneous incidence the model can predict the effect in strains with a higher spontaneous incidence.

These characteristics make it worth to explore the model in more detail, to apply the model to the analysis of animal and human data, to investigate the possibility to evaluate the parameters of the model, and to investigate the implications for risk evaluations at low doses and low dose rates.

d) Carcinogenesis Seminar
April 19 - 24, 1993 a seminar on Molecular Mechanisms of Radiation Mutagenesis and Carcinogenesis was organised at Doorwerth (NL), jointly prepared by CEC, DOE and RIVM.

Head of project 5: Prof.Dr. Von Sonntag

II. Objectives for the reporting period

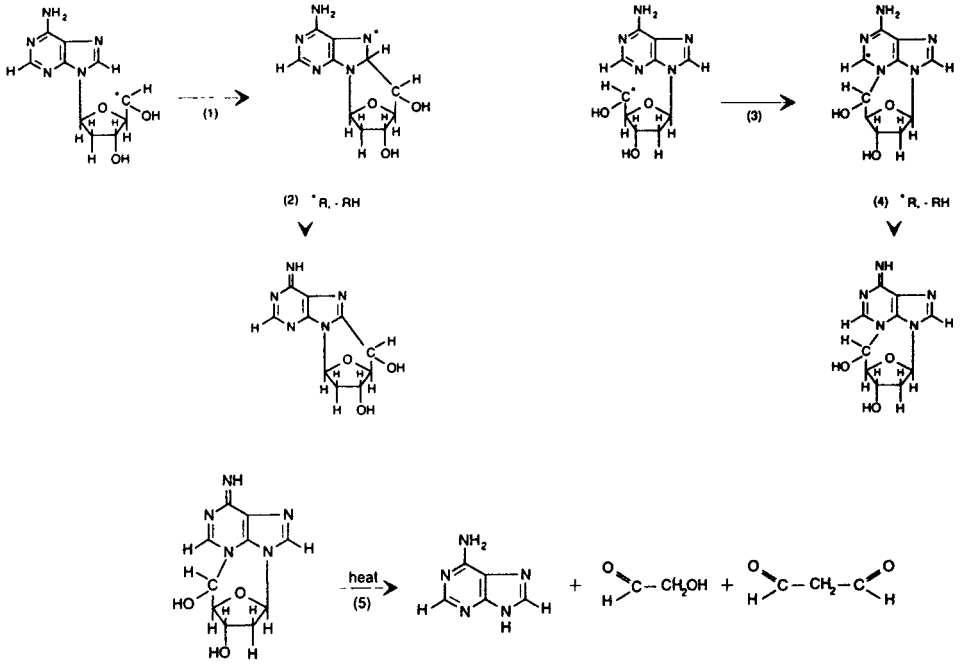
Radiolytic damage to the DNA in living cells may be subdivided into two components, the direct effect, and the indirect effect. In the former the energy of the ionizing readiation is absorbed by the DNA itself, in the latter by its environement, mainly water. Our present knowledge of the radiolysis of DNA is dominated by the reactions of water radicals with the thymine residue, and knowledge about radiolytic changes of cytosine and especially with the purine residues is rather limited. The programme has been started by investigating the radiation chemistry of the purine adenine in model systems such as 2'–deoxyadenosine, adenosine and the homopolynucleotide poly(A). In addition to the elucidation of the radiolytic products two important questions must be addressed: (i) is the release of unaltered bases due to an attack of the reactive OH radicals with the sugar moiety, or are there other routes to this important type of damage, and (2) are base radicals capable of transfering their radical site to the sugar moiety.

In the next period the investigation concerning purine radiation chemistry will be continued, since the work has not yet been completed. Special attention will be paid to the chemistry of purine radical cations. Since preceding studies have largely failed to yield identifiable products, this part of the project will be a difficult and risky one, but it will have to be attempted, because without any progress in this area major aspects of radiation–induced damage to DNA will remain obscure.

We also will start to investigate the chemistry of the direct effect. First preliminary experiments have already been carried out.

III. Progress achieved including publications

A detailed study on the radiation-induced damage to 2'-deoxyadenosine (AdR) is undertaken which has already yielded several remarkable results. When AdR (10^{-3} mol dm^{-3}) is γ-irradiated in N_2O-saturated aqueous solutions, OH radicals ($\underline{G}(^{\cdot}OH)$ = 5.5 x 10^{-7} mol J^{-1}) and H-atoms ($\underline{G}(H^{\cdot})$ = 0.6 x 10^{-7} mol J^{-1}) are generated as reactive intermediates. Among the products we have confirmed the previously reported products, viz. free adenine, adenine-derived formamidopyrimidine (A-Fapy), 8-hydroxy-AdR, and the 8,5'-cyclonucleoside. The formation of the latter is considered to be by way of the C(5') radical, generated by the OH-radical via H-abstraction. This radical then adds to the N(7) - C(8) double bond (reaction 1). In a subsequent step the N(7) radical thus formed disproportionates with other radicals present yielding the 8,5'-cyclonucleoside (reaction 2). We have now isolated an additional product whose ^1H and ^{13}C NMR data are compatible with the 3,5'-cyclonucleoside structure. Its formation may also start from the 5'-radical that now adds to the N(3) position (reaction 3). In a subsequent disproportionation reaction the 3,5-cyclonucleoside is formed (reaction 4). It is noteworthy that this compound gives a positive 2-thiobarbituric-acid test for "malondialdehyde" with 30% efficiency. To rationalize this reaction one may consider an acid hydrolysis of the 3,5'-cyclonucleoside followed by a retroaldol reaction (overall reaction 5). These results pose the interesting question whether reactions similar to those shown here contribute the so-called "malondialdehyde" found in γ-irradiated DNA.



In a further series of experiments the polynucleotide poly(A) was irradiated. Unaltered adenine and A–Fapy straight after irradiation as well as at various times after heating the samples to 95 °C were determined. The data are shown in Figures 1 and 2. It can be seen that some of the adenine is released during and/or immediately after irradiation, but there is much further release upon heating. The total G(adenine) = 1.3 x 10^{-7} mol J^{-1} is quite high, i.e. higher than expected for OH–attack at the sugar moiety, and the question arises whether there is more than one type of precursor. The trivial one would be OH–radical–induced sugar damage followed by immediate as well as delayed base release, similar to what we have previously observed with poly(U). With poly(A) we may also consider intermediate adenine hydrates that could lead to the observed effect. They may also be the precursors of the A–Fapy formed.

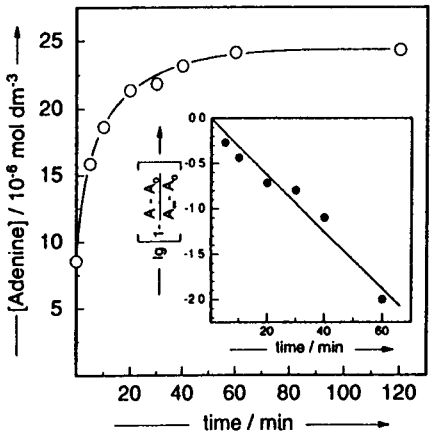


Fig 1

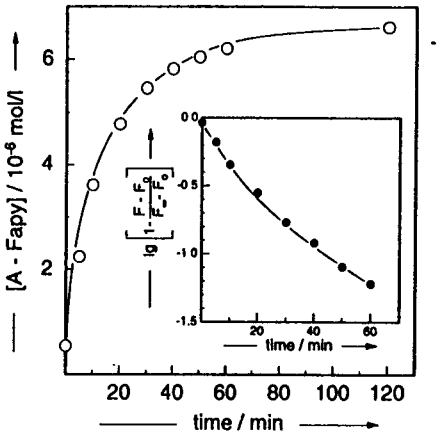


Fig 2

Figure 1. Instant production and subsequent release of the product A–Fapy upon heating at 95°C. Inset: the release reaction is not monophasic first–order (A = adenine concentration).

Figure 2. Instant production and subsequent release of unaltered adenine upon heating at 95°C. Inset: the release reaction is approximately first–order (F = A–Fapy concentration).

Head of project 6: Dr. Smith

II. Objectives for the reporting period 1.9.92 - 31.8.93

The intentions as set out in the previous report have been:

(1) to prepare multilayer unsupported organic films down to a thickness of ~3nm, in order to carry out low energy proton transmission measurements and
(2) to bring into operation an energy analyzed proton beam having energies in the range 1-10 keV, in order to make the above measurements.
(3) to begin the writing of Monte Carlo codes to model the interactions within the films and
(4) to continue to investigate the possibilities of using such thin films as dosimeters.

III. Progress achieved including publications

Good progress has been made on (1) and (2) above. Multilayer unsupported films 20 * 3nm thick have been produced over an area of ~60 mm^2 , and irradiated with an excessive current (50 uA/cm^2) of 50 keV protons to check their robustness. This has been shown to be satisfactory and to be well within the requirements for the lower intensity energy analyzed pulsed beam needed for the transmission measurements.
The electrostatic beam analyzer has been designed to achieve an energy resolution of 0.6% at 10 keV. It has been constructed and is now in the process of being aligned and tested. The same applies to the two detectors, one channeltron and one fast scintillator, which will be used for the transmitted and incident beams respectively.
Work has just begun on the adaptation of Monte Carlo codes to model the microdosimetric processes taking place in the films, and also on the analyzers needed to determine the charge states of the transmitted beam.
No further effort has been devoted to the dosimeter investigations because of lack of time.

Head of project 7: Dr. O'Neill

II. Objectives for the reporting period

The objectives were two fold based upon approaches involving DNA damage induction. Firstly, it was essential to establish the dependence of the induction of double strand breaks (dsb) in V79-4 and CHO cells on radiation quality using γ- and α-irradiation. Since α-radiation has been proposed to produce more severe damage due to the increased clustering complexity, the reparability of dsb induced in cellular DNA by α- and γ-irradiation, was determined as an indicator of damage severity as hypothesised from track structural approaches. The involvement of diffusible water radicals in DNA damage induction by both types of radiation was assessed. Secondly, *in vitro* studies on DNA damage formation in plasmid DNA have been initiated in order to gain experimental evidence for the involvement of clustering of damage.

These two approaches have established the foundations for probing in more detail the complexity of DNA damage and the influence of the chemical environment. The information is being used to develop models (with MRC(Phys)) to simulate the chemical processes occurring following the energy deposition events.

Future Objectives

The future objectives are to gain more insight into the influence of radical scavengers, e.g. DMSO, on the induction of DNA dsb by radiation of different qualities in order to gain a better understanding of the chemical stages in the development of DNA damage under normal cellular conditions. In order to assess the dependence of damage severity on radiation quality, the induction and reparability of dsb in V79-4 cells will be extended to aluminium soft X-rays, to assess the effects of electron track ends. Since the size of the critical target volume has been proposed to increase with the LET of the radiation, the technique to determine DNA-protein crosslinks under aerobic conditions will be developed. The significance of the complexity of DNA damage on various biological endpoints will be a major consideration of this period. The *in vitro* studies on damage induction in plasmid DNA will be focused on damage severity.

The information obtained from these studies will be continually fed into the development of more sophisticated models of radiation action and to include the chemical stages.

III. Progress Achieved, including Publications

These studies have been carried out in collaboration with MRC (phys) who provide the expertise for α-irradiations of mammalian cells and dosimetry.

(i) The effects of radiation quality on cellular DNA damage

The initial studies were undertaken to establish the RBE for the induction of dsb in V79-4 mammalian cells by α-particles using three different methods for the analysis of dsb, namely sedimentation, non-denaturing elution and pulsed field gel electrophoresis (PFGE). The induction of DNA dsb was determined in V79-4 mammalian cells following irradiation by ^{60}Co γ-rays or ^{238}Pu α-particles (average LET 120 kev μm^{-1}) under aerobic conditions.

The induction of dsb was found to be linearly dependent on the dose with the RBE for α-particles of 0.85 ± 0.14 (sedimentation) and 0.68 ± 0.12 (elution) compared with ^{60}Co γ-rays.

A discrepancy which has existed in the literature concerning the RBE for dsb induction by α-particles has been suggested to reflect the method of dsb determination. The method of PFGE for determination of dsb induction has been set-up in order to enable access to dose ranges which approach those used for cellular inactivation. Using the PFGE method [1,4], the RBE for induction of dsb in V79-4 and CHO cells by α-particles has been verified to be ~ unity at radiation doses of < 10 Gy. A possible discrepancy between these findings and those of Kampf and Eichorn is the centrifugation speed and the absence of proteinase k in their lyse solution so that the DNA may still be associated with protein.

The timecourse and extent of rejoining of radiation-induced dsb were determined over 3 hr period at 310K following irradiation of V79-4 cells under aerobic conditions. With low LET radiation, > 90% of the dsb are rejoined within the 3 h repair period. The ability of the cells to rejoin dsb induced by α-irradiation is significantly reduced with only 30-50% rejoined within 3 hr. With low LET radiation, the kinetics of rejoining is distinctly multi-component whereas with high LET radiation the majority of dsb rejoining occurs within 1 hr. It is inferred that the yield of residual damage reflects the lesion severity whereby it has been hypothesised that the clustering of damage from the energy deposition events increases with increasing LET of the radiation (MRC (phys)). Therefore different types of dsb are produced whereby chemical modifications of the DNA in the vicinity of the breaks may influence their subsequent reparability. With α-irradiation, the proportion of these more complex damages is greater than those produced by low LET radiation [1,2].

The RBE for cellular inactivation at the 10% survival level was determined to be ~ 5.3 a value that is comparable with that obtained from comparison of the residual yields of dsb. From the D_0-value of 0.68 Gy for α-irradiation, the average number of tracks which pass through the nucleus is calculated to be 4.7 tracks/lethal lesion. Therefore the average number of tracks which pass through the cell is significantly greater than the average number of lethal lesions per cell. Further, based upon the residual yield of dsb at 3 hr it is estimated that ~ 7 residual dsb/cell nucleus/lethal event, are produced for α-irradiation.

(ii) The role of diffusible radicals in DNA damage induction

Preliminary studies have been undertaken using water radical scavengers in order to assess the 'scavengeable' yield of radiation damage based upon dsb formation and cellular inactivation by γ- and α-irradiations. These approaches should shed light on the influence of the chemical environment around DNA in cells and the involvement of water radicals in influencing the complexity of damage with radiation quality. Dimethyl sulphoxide (DMSO) was chosen as the water radical scavenger. Prior to determination of DNA dsb, it was necessary to establish the effect of additives upon the ability of the cells to remain as monolayers using confocal microscopy (S. Townsend). The effect of DMSO upon V79-4 cells as monolayer was found to cause the cells to round up from a cell thickness of ~ 3 μm to thickness of ~ 14 μm but still remain attached. The optimum concentration of DMSO was found to be 0.5 mol dm^{-3} since at higher concentrations, the cells detached. From previous studies with low LET radiation on the protective effect of DMSO, cellular inactivation, a lower concentration limit of DMSO of < 0.2 mol dm^{-3} was shown to have minimal effects.

The induction of dsb in V79-4 cells by γ-radiation was reduced by 55 ± 3% in the presence of 0.5 mol dm^{-3} DMSO. Preliminary indications [1,3] show that DMSO also protects against the induction of dsb by α-particles but to a less extent. Since DMSO changes the morphology of the cells, we are presently investigating whether this protection by DMSO with α-particles is due to chemical scavenging and/or dose attenuation. The scavenger, 2-methyl-2-propanol (t-BuOH), which is ~ 10 less effective at scavenging water radicals will be used as the control. At a low concentration of 0.1 mol dm^{-3} of t-BuOH, the morphology of the cells is similar to that in the presence of DMSO. From the effects of t-BuOH, it will be possible to comment on the chemical protective effect with DMSO. At this stage, it is apparent that the role of diffusible water radicals at inducing cellular DNA damage becomes less at higher LET. Whether this reduction is a reflection of less radicals diffusing to the DNA due to intra cluster recombinational events remains open to question. In order to address the role of diffusible radicals in cluster damage severity, it is planned to assess the effect of DMSO on the residual yield of dsb in the case of α-irradiations. The question arises as to whether the dsb which are prevented by DMSO correspond to the class of dsb which are rejoined following α-irradiation and probably represent less severe damage.

(iii) Complexity of DNA damage produced *in vitro*

The enzyme S1 nuclease was used to probe for complex damage induced *in vitro* through irradiation of plasmid DNA in the presence of 1 mol dm^{-3} ethanol in order to limit the range of diffusion of water radicals. This condition is thought to mimic the cellular environment with respect to diffusion. Initial findings indicate that with low LET radiation there is little evidence for severe damage.

Since the yield of water radicals which escape the clusters is reduced upon increasing the LET, this was confirmed using plasmid DNA. In the absence of scavenger, the yield of diffusible radicals from water is reduced as indicated through less DNA damage at high LET. These studies will be extended to the effect of hydration on DNA on the dsb:ssb ratio as an indicator of damage complexity and whether S1 nuclease sensitive sites are produced by α-particles.

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Progress report

Contract: **FI3P-CT920041** **Sector: B11**

Title: Specification of radiation quality at the nanometre level.

1	Colautti	INFN - Legnaro
2	Watt	Univ. St.Andrews
3	Harder	Georg-August Univ. - Göttingen
4)	Leuthold	GSF - Neuherberg
4	Izzo	Univ. Roma - Tor Vergata
6)	Kraft	GSI - Darmstadt

I. Summary of Project and Global Objectives

The common aim of this international research group is "research concerning the best possible way of specifying radiation quality for radiobiology and radiation protection by a single physical parameter expressing the track structure". The quoted definition of the group's aim can be easily recognised as an old but urgent target because the application fields of radiation protection and radiotherapy require a physical parameter which can predict its biological efficiency. That aim is difficult because the parameters studied in the past, especially the unrestricted LET and the lineal energy y for a reference volume size of about 1μm, have been proven to be too "rough" to play a role as indicators of track structure at nanometre level. But nanometer is the scale of the critical target of radiation effects, as both molecular biology and radiobiological analysis suggest.

The renewal of this old aim was motivated by new ideas which appeared to describe more adequately the meaningful track parameters at nanometre level (restricted LET and linear primary ionization) The research on the gas detector physics allows to design microdosimetric instruments able to measure ionization distributions near the particle track with nanometre resolution and the recent advances in technology suggests the possibility to develop a nano-dosimeter in condensed phase. Modern Monte-Carlo codes, are able to simulate the interaction of particles with the matter at nanometre level. This supports both experimental and theoretical research.

The research group, been enlarged in the meantime by the experimental experience of ESR local radical concentration measurements of Roma group, with the new electron measurements calculations by the Darmstadt group, is going to renew that aim in the light of the achieved results.

Head of Project 1: Dr.Colautti

II Objectives for the reporting period

In order to perform experimental microdosimetry at nanometre level with gas proportional counters (CPC), it is necessary to employ low pressures and thin anodes. CPC works in these conditions at very high reduced electrical fields and its gradient is very high., consequently the electron swarm is not in equilibrium with the electrical field and his behaviour cannot be predicted by the classical Townsend theory. The Legnaro research group of radiation physics has developed an experimental set-up to study the work characteristics of a cylindrical proportional gas counter (CPC) at low pressure (0.2-6.0 torr). Low-energy nitrogen beam has been used as a probe inside the gas detector. The beam scans almost all the sensitive volume between the cathode and the anode, parallel to the equipotential lines. The reduced first ionization coefficient, α/N, can be extracted from the gain data at different radial distances. Experimental measurements of α/N in tissue-equivalent gases will allow for designing new microdosemeters working at very low pressures.

The effort to develop a gas track detector able to measure the radial ionization profile around a charged particle track with nanometre resolution will continue. The detector is basically composed of two parts: a small drift region in which the particle travels producing ionisation in the counting gas, and a multiplication region in which the electrons are multiplied to give a detectable signal. The two regions are connected through a narrow slit which determines the detector sensitive volume: only the electrons under the slit are measured. The variation of the beam-slit distance allows to measure the radial ionization yield.

First results point out the necessity i) to use UV laser beam as a probe to produce ionization lines moveable inside the detector to check the shape of the sensitive region; ii) to measure the electron diffusion contribution to the experimental data uncertainty; iii) the opportunity to substitute the proportional counter used to multiply the electrons with a single electron counter made with a multiwire multistep proportional chamber (MSC).

A new measurement chamber will be designed to allow for all the new tasks. The detector will be modified to measure single electrons. This task will be achieved making the electron to drift in the multiplication region after the slit, for a distance long enough to separate them in time. The MSC is a fast detector ($\leq$1ns), then the avalanches produced by the electrons will be detected separately by a fast digitizer. In such a manner it should be possible really "to count" the electrons produced at a given distance from the particle track. The MMPC will be manufactured in collaboration with the Weizmann Institute (Israel) which developed originally this kind of detector.

III Progress achieved including publications

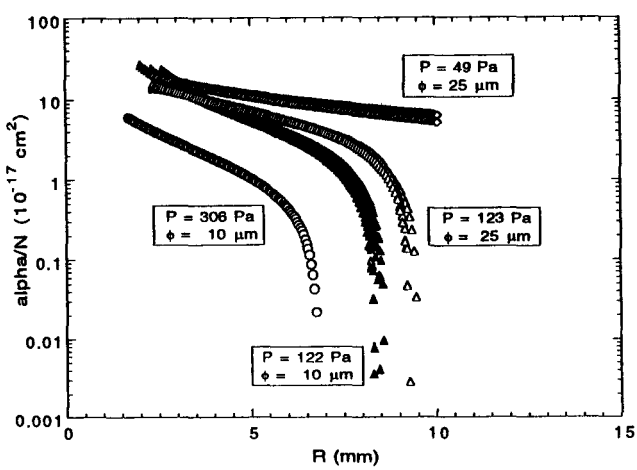


Figure 1

Extensive measurements of α/N have been done in CH_4, Ar-CH_4 90/10 mixture and in propane based tissue-equivalent mixture. Microdosemeters are proportional counter with central electrical field, therefore the field strength gradient is always relevant and it influences the electronic avalanche. At low pressure other non-equilibrium phenomena occur which are still under investigation. An example is in figure 1 where the variation of α/N, inside a cylindrical microdosemeter filled with pure methane gas, is plotted towards the distance from the anode. The cathode wall is at 10.2 mm. All the curves are at the same value of the ratio $\Delta V/\ln c/a$, where ΔV is the voltage difference between the anode and the cathode and a and c their respective diameters. The strength field gradient is then equal for all the curves at the same R. The avalanche limit is detectable as the sudden falling down of the curve towards the zero value. Decreasing the pressure the avalanche limit shifts towards the cathode wall, according to the fact that the reduced electrical field strength increases at the same R. At very low pressure the avalanche occupies all the sensitive volume, eventually (curve at 49 Pa). Field grids properly designed could constrain the avalanche in a defined volume allowing for a gain high enough even at low pressures. Secondary non-equilibrium effects, relevant in these conditions, don't allow for a simple parametrisation of gain and α/N curves. In fact figure 1 shows that curves taken with two different anode diameters, but with the same field gradient and pressure, are surprising different. Experimental studies will continue in order to optimise the design of future nanodosemeters.

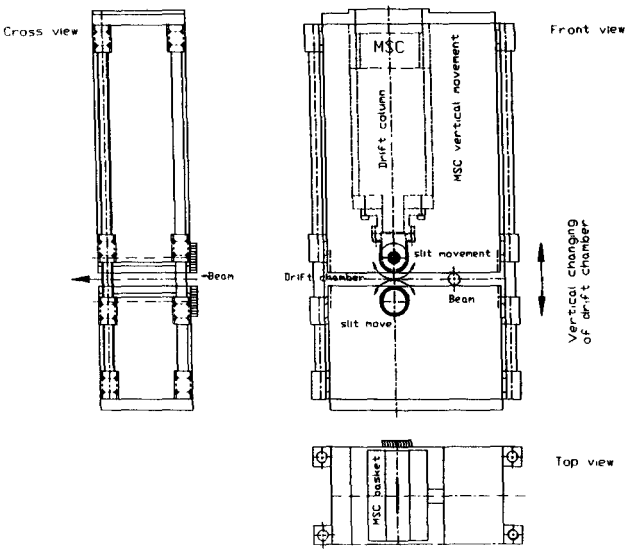


Figure 2

The ionisation distribution across an α-particle track has been measured with a track detector slightly modified to minimise secondary electron emission. The influence of parasitic δ-ray-induced secondary electron emission was estimated and though it is limited to about 7% or less, it could be further reduced by a more adequate choice of the detector geometry and materials. A fundamental limit of the described approach is due to the fact that ionising particles deposit in nanometric volumes only a few ionisation electrons. Under these conditions the pulse-height distributions suffer considerable statistical fluctuations which may mask the basic radiation phenomena which we want to study. To overcome this limit we have planned to substitute the cylindrical proportional counter with a multistage avalanche detector (MSC), capable of counting with high efficiency pulses induced by single electrons down to about 100 Pa. In this way we should be able to measure directly the ionisation by counting the number of electrons created in the sensitive volume. The draft of the new track detector is in figure 2. Electrons created in the sensitive volume, as defined by the drift chamber height and slit dimensions, will first drift in the drift column and then detected one by one with the MSC

counter. The new reaction chamber has been manufactured and is ready to house the new track detector which will be manufactured next year.

P.Colautti, V.Conte, G.Talpo, G.Tornielli and M.Bouchefer
A Method to Investigate the Working Characteristics of New Microdosimetric Detectors which Use Low Gas Pressure
Nuclear Instruments and Methods, NIM A 321, 392-402(1992).
P.Segur, P.Colautti, V.Conte, G.Talpo and G.Tornielli
Comparison Between Experimental and Theoretical Determination of the Space Variation of the Gas Gain Inside Low-Pressure TEPC's
Radiation Protection Dosimetry to be published (1993).
P.Colautti, G.Talpo, G.Tornielli, A.Akkermann and A.Breskin
Measurements of Ionization Distributions Around a Particle Track at a Nanometre Level.
Radiation Protection Dosimetry to be published (1993).

Head of project 2: Dr. D. E. Watt

II. Objectives for reporting period. 1992-1994.

1) *Biophysical modelling of radiation effects for radiation protection.* Main objectives are to extend the St. Andrews model to include radical action and to determine the extent of its validity; to appraise and criticise this model in the context of the many other models and to explore the applicability of models to 'unified dosimetry' for radiological protection Key physical parameters to be investigated for expressing the quality of track structures in a 'unified' way are 'the mean free path for primary ionisation' and the 'dose-restricted LET'.

2) *Biological 'benchmark' data base.* Data base to be extended to include published results on transformation; mutations and incorporated radionuclides (Auger electron and alpha particle emitters) - in addition to the more usual end-points of survival and chromosome aberrations. Data to be applied to interpretations in objective (1) above.

3) *Track structure parameters in liquid water.* To provide information on track structure parameters for interpretation of effects. Tables of track-structure parameters for liquid water to be prepared for all radiation types using recent ICRU information. The objective is to provide a consistent data set and to include parameters not readily available in the literature for equilibrium spectra e.g. mean free paths and restricted LET.

4) *Absolute dosimetry in the condensed phase.* Consideration to be given to the possibility of producing devices in the condensed phase and with nanometre dimensions to simulate the DNA for absolute dosimetry.

III. Progress achieved including publications.

1. *Biophysical Modelling:* For calculation of radiation effects at low doses near environmental levels it is necessary to model both 'direct' and 'indirect' effects along single charged particle tracks in the equilibrium spectrum generated by the radiation field. A quasi-empirical approach has been used here to extend the previous model for 'direct' action to include the 'indirect' i.e. diffusing radical component of damage to the DNA in mammalian cells. First a general semi-empirical expression was determined for the transition in damage from solid to liquid phases at different concentrations of enzyme targets (known to obey single-hit, single-target kinetics); different qualities of radiation; different dose-rates and for the presence of radical scavenger. [In pursuing these studies, the need for more experimental data for a wider range of low and high -LET quality was identified]. From the results information was obtained on the role of dose-rate; on diffusion lengths, on the type of radical predominantly responsible for damage ($OH^{\cdot}$) for the inactivation. Assuming that water radicals are the main cause of 'indirect' damage in mammalian cells it is a simple step to deduce from the enzyme results the probability of induction of single and double strand breaks in the DNA by making the additional assumption that basically the same radical kinetics are involved and then applying Poisson

probabilities to determine the effect in mammalian cells. The resulting model is complete and is currently undergoing validity tests against published data on the yields of double-strand breaks in intracellular DNA (1,2). Testing of the model will be by June, 1994.

Plans are well advanced to compare critically the St. Andrews model with the many other models proposed. A general review of all important models is well advanced and these will be tested against a selected set of common bench mark data to determine their virtues and limitations wherever possible. This work is also expected to be completed by June, 1994.

In preparation for the outcome of this, the methods used by ICRP to determine risk coefficients are being examined to explore the consequences for the permissible levels of radiation should a revised model of damage be incorporated.

As part of the need to test the value and limitations of models, the work has been applied to a number of unusual situations where conventional dosimetry fails e.g. to oncogenic transformation in mammalian cells (5); resonance absorption of monochromatic synchrotron radiation which results in Auger electron cascades (8,12,13) and to an assessment of the components of radiation action by relativistic nuclei (7). In the latter it is found that the low energy high-LET fragments are the major contributors towards the localised dose, to e.g. astronauts, and that the fragments formed as a result of 'stripping' of one to three nucleons from the target nuclei (typically boron, carbon and nitrogen nuclei) by an incident proton are the most damaging. The direct ionisation effect due to an incident 1GeV protons is small.

There appears to be good prospects of developing a 'unified' system of dosimetry applicable to both external and internal irradiations. It would be based on the fluence of charged particles in equilibrium with the radiation field and a physical parameter descriptive of the single track 'core'.

2. *Benchmark data-base.* Compilation of a formal data-base of survival, transformation, mutations, incorporated radionuclides and strand breaks in DNA is well advanced and will be completed up-to-date by June, 1994.

3. *Track structure parameters in liquid water.* In the previous contract, physical track structure data were determined for electrons and photons. Extension of this work to 72 types of heavy particles ranging from protons to uranium ions at energies from 100eV to 1 GeV is now complete. Values are tabulated for delta ray maximum energies and ranges and for their mean values assuming an appropriarte delta ray spectrum shape. Other parameters listed are energy per unit mass; β^2, z^2/β^2, linear primary ionisation , mean free path, LET, track and dose restricted LET_{100}, ranges and W values in water. Tables of data are given for both instantaneous energies and for the average values in the charged particle equilibrium spectrum. (3).

The final results for the track structure quantities for neutron-generated recoils (Hand O) are complete and are being published as a University Report. Parameters listed are recoil source densities, partial kerma factors, euilibrium fluences, maximum recoil energies and ranges, average and maximum delta ray energies; W values; β^2, z^2/β^2, LET, $L_{T,100}$ and $L_{D,100}$, variances, microdose quantities - y_F and y_D, linear primary ionisation, and mean free path for ionisation (4). This completes the trio of data tables for all major radiations and enables modellers to have a ready reference to the

relevant physical quantities.

4. *Detectors for absolute dosimetry.* Damage models are of value in several ways e.g. for interpolation/extrapolation of biological effects; for improving our understanding of damage mechanisms and for guidance in the design of the required response of instrumentation. However, with regard to the latter, if the nature of the sensitive sites is known then an instrument designed to have the same response would in effect be an absolute dosimeter and it would be unnecessary to know the type, quality or intensity of radiation field to assess the effectiveness. This would not depend on a damage model. Currently it seems to be generally agreed that the main sensitive sites in mammalian cells is the DNA and that double-strand breaks in the DNA constitute the significant lesion. On this basis, the problem of absolute dosimetry reduces to the production of a device that can simulate the response of the DNA i.e. a device that has sensors of nanometre dimensions distributed in a similar way to the mean distribution in cells. A double coincidence within the two nanometre site may correspond to a double strand break in the DNA. Exploratory studies have been made on various types of detector which it is thought offer the potential to simulate the response of the cellular DNA.

Initial experiments have been performed with MOSFET devices which are being operated in a new configuration. In the first instance measurements have been made of the five characteristic modes of operation of various semiconductor devices having different configurations and doping materials (p- and n-type), obtained from commercial suppliers. It was found that the n-type MOSFET responded encouragingly well to alpha, X- and gamma-radiation exposures. An exciting feature of the new system is that it has the ability to distinguish between different radiation types. The source-drain current was found to be a linear function with respect to the drain voltage and corresponded to the dose-rate delivered. A method has been developed to ensure that the signal can be accurately reproduced in successive measurements. In these exploratory experiments, the intrinsic efficiency of the devices is close to 100%. In principle the devices have single charge sensitivity and can be produced in arrays. The sensitive region is the depletion depth which is controlled by the gate voltage and has dimensions of nanometres.

Other exploratory work is being pursued with scintillators spheres of micron dimension and liquid filled miniature detectors.

III a. Publications

1. Observed cellular effects lead to a track 'core' model of radiation action. D.E.Watt. Workshop on 'Biophysical modelling of radiation effects'. Padua, Italy. Sept. 2-7, 1991. Chapter 7. Adam Hilger, 1992.

2. An empirical model for induction of double strand breaks in DNA by the 'indirect' effects of ionising radiation. D.E.Watt and S.J.A. Hill. 11th Symposium on Microdosimetry. Gatlinburg, Tennessee. Sept. 1992. Radit.Protect.Dosim. (to be published) 1993.

3. Track structure data for ionising radiations in liquid water. Part 2: Heavy charged particles, 100eV to 1GeV. D.E.Watt. University Report: biophys/1/93. University of St. Andrews, Scotland, UK. KY16 9SS.

4. Track structure data for neutrons (1 keV to 20 MeV) in liquid water. D.E.Watt. University Report: Biophys/9/93. University of St. Andrews, Scotland, UK. KY16 9SS.
5. Comment on radiation effects with special reference to transformations. D.E.Watt. Letter to the Editor. Int.J.Radiat.Biol. Vol. 61, (2), 263-267, 1992.
6. Yields and quality of photoneutron fields in the vicinity of high energy therapy machines. 11th Symposium on Microdosimetry, Gatlinburg, Tennessee. Sept.,1992. Radiat. Protect. Dosim. (to be published) 1993.
7. Assessment of components of radiation action by relativistic nuclei. D.E.Watt and J. Storey. Workshop on 'Biological applications of relativistic nuclei', (BARN) Clermont-Ferrand (France), October 14-16, 1992.
8. Comment on the damage induced by monoenergetic X-rays at the resonance absorption energy of intracellular phosphorus in yeast cells. 4th Intnl. Conf. on Biophysics and Sunchrotron Radiation. Aug. 30th-Sept 5th, 1992. Tsukuba, Japan.
9. Preparation of A-150 tissue-equivalent plastic films. E.B. Saion and D.E.Watt. Phys.Med.Biol. 37, (12),2303-2308, 1992.
10. Calculation of effective stopping power of ions generated by neutrons in tissue constituents. Petranika 15, (1), 55-59, 1992.
11. ICRU Report 49. Stopping powers and ranges for protons and alpha particles. Berger, Inokuti, Andersen, Bichsel, Powers, Seltzer, Thwaites and Watt. ICRU, Bethesda, Maryland 20814, USA.
12 Analysis of damage induced by monochromatic synchrotron X-rays in bromine of DNA due to Auger processes. K. Kobayashi, T.Jin, N.Usami and D.E.Watt. Submitted to Nuclear Techniques, China.
13 Damage induced by monoenergetic X-rays at the resonance absorption energy of intracellular phophorus in yeast cells. T. Jin, D.E.Watt and K. Kobayashi. Appl. Radiat. and Isotop.: Intl. J. of Radiat. Applic. and Instrumentation, Part A. Sept., 1993.

Head of project 3: Prof. Harder

II. Objectives for the reporting period

The study of the regularities of track structure, together with Neuherberg, St. Andrews and Darmstadt, will be completed. Especially the general validity of the theorem of the independence from ion type and ion energy of the low-energy delta-ray contribution to the nanometer-scale energy deposition pattern will be further substantiated. Since restricted LET turns out as the appropriate parameter of radiation quality, complete tables of restricted LET will be produced for various ions, and the concept of restricted LET will be utilized in finalizing the attempt to explain the δ-ray "hooks" of heavy-ion survival and action cross sections. Further experimental data of such cross sections will be correlated with restricted LET. Our group will contribute the theoretical part in a study of delayed fluorescence in liquid scintillators due to the LET-dependent triplet-triplet interaction, together with Rome.

III. Progress achieved including publications

Studies of track structure and its regularities, especially of the above mentioned independence theorem, have been completed for protons and alpha particles, and the importance of restricted LET for the nanometer-scale energy deposition pattern has been proven. Tables of the mean values of restricted LET, including the δ-ray terms, have been devised for these ions. It has been possible to fit further chromosome aberration data for protons and heavier ions into the already existing restricted LET dependence. The triplet-triplet interaction project has not yet been started. Results have been published in three symposia, see additional page.

List of publications (Goettingen)

1. **Theory of pairwise lesion interaction**
   D. Harder, P. Virsik-Peukert and E. Bartels
   Biophysical modelling of radiation effects, pp 179-184 (1992).
   Edited by K.H. Chadwick, G. Moschini and M.N. Varma.
   Adam Hilger, Bristol, Philadelphia and New York.

2. **Restricted LET determines the yield of intratrack pairwise lesion interaction**
   D. Harder and E.R. Bartels
   Radiation Research - A Twentieth-Century Perspective, pp 427-432 (1992).
   Edited by W.C. Dewey, M. Edington, R.J.M. Fry, E.J. Hall and
   G.F. Whitmore. Academic Press, Inc. .

3. **Theory of intra-track pairwise lesion interaction**
   D. Harder, R.P. Virsik-Peukert and E.R. Bartels
   accepted for publication in Radiation Protection Dosimetry.

Head of project 4: Dr. Leuthold

II. Objectives for the reporting period

1. Implementation of a computer program for the calculation of double differential ionization cross sections under proton impact and extension of the Monte-Carlo code up to 100 MeV proton energy.

2. Calculation of proximity functions and dose mean lineal energy for protons up to 100 MeV.

III. Progress achieved including publications

1. The computer code "DXBT" for the calculation of double differential ionization cross sections written by B. Senger (B. Senger, Z. Phys. D- Atoms, Molecules and Clusters 9, 79-89 (1988)) was made available by the author in form of a copy of the program source text, was typed in, and runs now on our computer system.
   This code allows the calculation of double differential cross sections under proton and alpha particle impact up to 100 Mev. Additionally, as the author has shown, other gases than water vapour can be treated. Figure 1 show the double differential ionisation cross sections for 100 MeV protons in water vapour.
   Accordingly, the Monte-Carlo code for the simulation of charged particle tracks and the analysis of particle track structure was also extended up to 100 MeV. The excitation cross sections are derived from stopping power cross sections assuming a mean excitation potential of 12.6 eV.

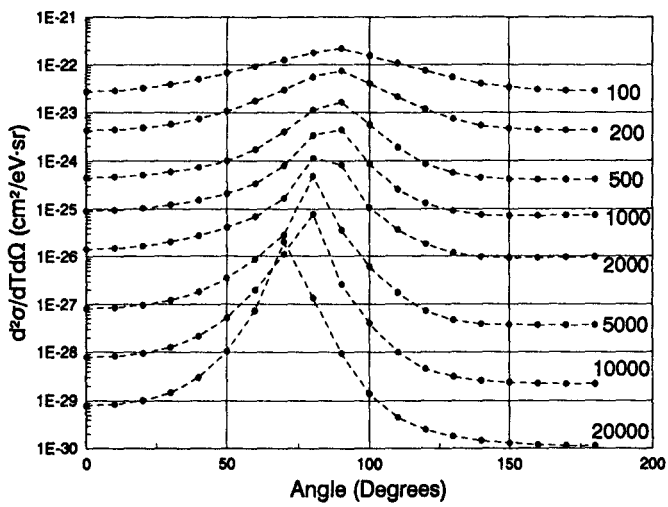


Fig 1: Double differential ionization cross sections as function of the emission angle of the electrons for 100 MeV protons. The parameters on the right side give the energy of the emitted electrons in eV.

2. The analysis of particle track structure was extended up to 100 MeV protons in water vapour. The access via the proximity functions give the possibility to distinguish different structural components of the particle track. From the proximity functions the dose mean lineal energy $\overline{y_D}$ was calculated. Figure 2 gives the result for 100 MeV protons in spherical targets of 1 nm up to 100 nm diameter. The dominance of the contribution of single electron tracks (e-component) due to the event clustering at their track ends is clearly seen,whereas the contribution of the correlation of different electron tracks (e-e component) is diminished due the increased distance of their production. The LET-approximation largely underestimates the dose mean lineal energy (s) at nanometer target diameters.

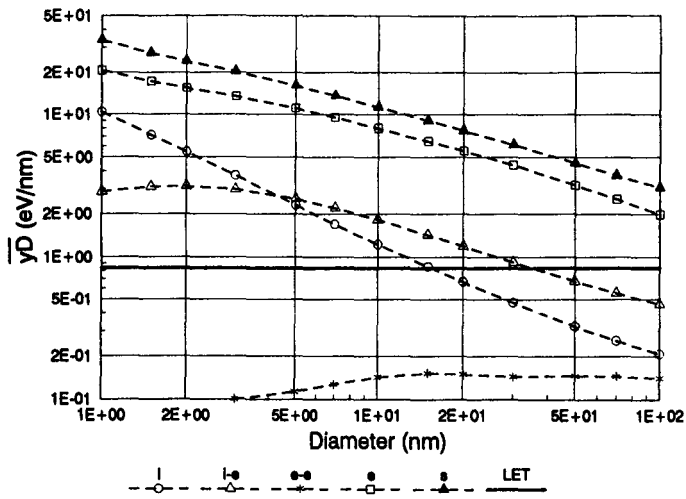


Fig 2: Dose mean lineal energy $\overline{y_D}$ as function of sphere diameter for the different structural components, the sum (s) of them and the LET-approximation.

Acknowledgement: I thank Dr. B. Senger for his help and assistance.

Head of project 5: Dr. Izzo

II. Objectives for the reporting period

The main objective for the reporting period was to improve the methods of study of track structure based on ESR techniques, so that they can be used consequently in the study of tracks in various targets (nucleosides, nuclear acid bases, dry and wet DNA), generated by γ rays as well as by light and heavy ions. The radicals associated with the track are formed in clusters resulting in the difference between the local and mean radical density. The distances between the radicals and sizes of the clusters are directly related to the track parameters. These characteristics can, in principle, be found by ESR measurements and the other objective was thus to improve the existing ESR spectrometer to make this study feasible.

In the next period of work a study will be made on the track structure in above mentioned nuclear components, including few varieties of DNA , irradiated with heavy ions. Use will be made of experimentally found local radical densities and of build-up curves of radicals and their clusters, to determine the sizes of clusters (spurs) and link them to the calculated track parameters. From the yields of radicals from DNA and water we shall estimate the contribution of direct and indirect radiation effects.

In collaboration with Göttingen and St. Andrews a feasibility of a condensed phase detector capable of measuring the unified radiation quality and dose, based on molecular photonics or nanoelectronics will be studied.

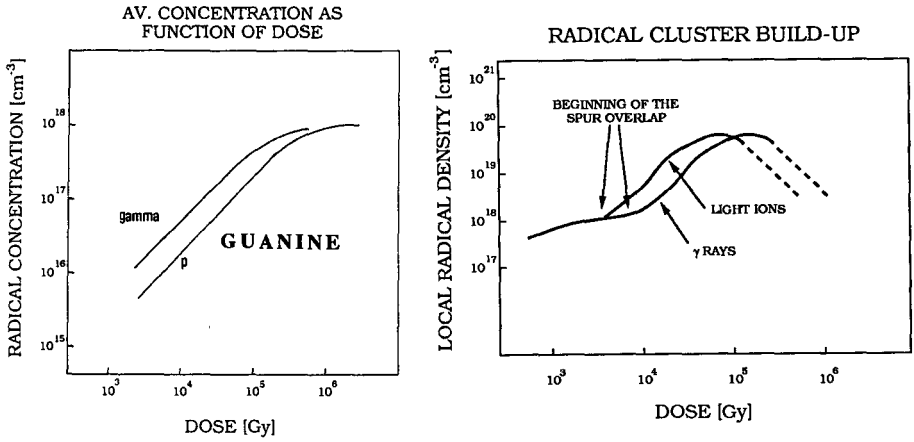
III. Progress achieved including publications

a. The measurements of track structure by ESR are done *post mortem* i.e. after almost all processes following the passage of an ionizing particle have run its course, except the slow radical diffusion and decay. The radicals are observed forming clusters, evidenced by a difference between the local radical density and the average value. The closeness of spins broadens the spectral lines and affects relaxation times of radicals.

For the low LET radiations the mean spacing between the radicals inside the spur is $\bar{d} = kc_{loc}^{-3}$, where k is a coefficient depending upon the shape of the spur and c_{loc} is the local concentration. The values of k calculated by Monte Carlo method for some probable spur shapes are in the range 1 - 1.5 . The measurements of c_{loc} were performed on adenine, cytosine, thymine and guanine and some of their corresponding ribosides and also on few varieties of DNA from herring sperm, from salmon sperm, from calf thymus and from *Xenopus* eggs. A nucleoprotein, protamine was also included. All the materials were carefully characterized with respect of purity, water content and in case of DNA the degree of degradation was also monitored. The samples were irradiated in vacuum at ca. 90 K by ^{60}Co γ rays in the range of doses 100 Gy - 100 kGy or by a 32 MeV proton beam in the dose range 15 kGy - 400 kGy . The average radical concentration, read at 140 K, as a function of dose follows a known sublinear law (Fig. 1). The local radical densities were determined by two methods: continuous saturation (fast and slow passage) and dipolar linewidth. Most of the saturation curves were interpreted as cases of nonideal inhomogeneous saturation and the spectra were decomposed into their Lorentzian and Gaussian components by specially written software. The local densities were also obtained from the linewidth measurements using the formula $c_{loc} = 1.85 \times 10^{23} \Delta H_{dip}$, where

ΔH_{dip} is the dipolar linewidth. The linewidth method of determination of c_{loc} gave results which agree to within ± 8 % with those found by continuous saturation, the difference being larger for the higher doses. Although the linewidth method appears simpler, for complex spectra of multiple radicals, like in some DNA samples, the continuous saturation method seems easier.

The ratio of local to average radical densities $\varsigma = c_{loc}/c_{av}$ diminishes with the dose. A typical for the materials studied by us $c_{loc} = f(D)$ dependence for 1.25 MeV γ rays and for 32 MeV protons is shown schematically in Fig. 2.

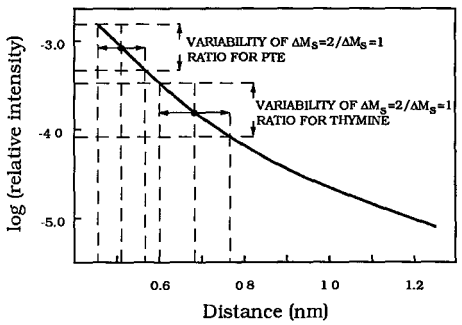


The isolated spur region exists if $\varsigma \gg 1$. Within the corresponding dose range in the ideal case c_{loc} should not change with dose, but in fact changes slowly until the individual spurs are starting to overlap. For the low LET radiation this takes place at doses of about 5 - 10 kGy where c_{loc} starts rising again. At still higher doses the c_{av} approaches c_{loc} and begins the process of radiation stimulated radical recombination, known already from radiation chemistry of polymers.

The radical spacings in the spur are of an order of units, tens and hundreds of nanometers. The spur model of accumulation of radicals appears to be valid as long as the mean free path between the primary ionizations is larger than the spur dimensions. On a smaller geometrical scale of an order of tenths of a nanometer there are known radical pairs, which are two-component clusters with radicals forming a triplet. There are considerable difficulties in studying radical pairs in nuclear biochemicals because one should reach the liquid helium temperatures to irradiate and measure properly the concentrations with good accuracy. In our laboratory we can only reach liquid nitrogen temperatures. Formation of radical pairs was studied in samples of polyethylene (PTE) treated thermally to reduce its crystallinity in order to weaken the modulating effect which the crystal lattice has on the spatial distribution of components of a pair. Polyethylene was treated as a model compound because its radicals and radical pairs can be studied at LN_2 temperature and because its crystallinity can be significantly modified by simple means. Pairs were also found in some nucleosides and nuclear acid bases, particularly in thymine. The spacing between the radical components was determined by observation of

the splitting in the spectrum as well as from Eatons' formula which deduces the spacing from the intensity ratios of full field and half field transitions ($\Delta M_s=2/\Delta M_s=1$).

It was originally suggested by us that the yield of radical pairs and the spacing of components may depend on such radiation characteristics as LET, restricted LET L_{100} or mean free path between ionizations. With a comparison limited at present to two radiations, ^{60}Co gamma rays (LET 0.25; keV/μm) and track segment of 32 MeV protons (track averaged LET 3.5 keV/μm) there were prominent differences of pair yields but no change in the pair spacing was reliably observed (Figure 3)



DEPENDENCE OF RATIO OF INTENSITIES
OF THE HALF-FIELD/FULL FIELD TRANSITIONS
ON THE INTERSPIN DISTANCE

A search was made in the spectra of samples irradiated to the maximum dose for the continuum expected from formation of random, non-geminate pairs, but no evidence of such clusters was found.

Modifications and improvements to the existing ESR spectrometer (Bruker ED 200) were : development of variable temperature accessory cooled with vapours of liquid nitrogen, interfacing of a computer for data storage and manipulation, development of a system for pulverizing tablets containing target material and transferring the powder to the sample tubes without warming up of the material.

In conclusion it is possible to say that ESR study of radical densities even in its early stage of development, brings additional information on the track structure in nuclear biochemicals.

Publications:

1. Ettinger K.V. and Hołowacz J. *On the Magnetic RF Field in Some Resonant Systems Used in the Local Density Determination by Saturation Method.* Applied Radiation and Isotopes, 44.373-376.1993
2. Izzo G., Ettinger K.V. and Hołowacz J. *ESR Windows onto the Nanometre Landscape.* presented at the Eleventh Symp. on Microdosimetry, Gatlinburg 1992 and submitted to Radiation Protection Dosimetry.
3. Izzo G. and Hołowacz J. *Post Irradiation Features of Radiation Field Measurable by ESR in Polyethylene*, presented at the VIIth Meeting of the Italian Society for Radiation Research, Capri 1992 and submitted to Acta Physica Medica.

Head of project 6: Dr. Kraft

II. Objectives for the reporting period

1.) Analysis of the δ-electron spectra taken in heavy ion atom collisions in gas targets (see attached list).
2.) Preparation for and measurements of electron emission from more complex molecular targets SF6, H_2O etc.
3.)Monte Carlo calculations of ionization deposition at small distances from the heavy ion path using the improved experimental double differential δ cross sections.

III. Progress achieved including publications

1.) Measurements with different heavy ions on various gas targets including energy and charge state variation have been completed (see attached list).
2.)Monte Carlo calculations of ionization and energy deposition in volumes having diameters smaller than 5 μm at distances down to a few hundred nanometers from the particle path have been performed. They sre in good aggreement with all existing experiments.

Publications:

M. Krämer, G. Kraft: Hoavy ion track structure calculations, in: Biophysical Modelling of Radiation Effects, Adam Hilger 1992, 61-68

U. Ramm, O. Jagutzki, G. Kraft, H. Schmidt-Böking: δ electron emission from heavy ion atom collisions, Radiat. Eff. Def. in Solids 1993, 126 73-76

M. Krämer, G. Kraft: Track structure and DNA damage, Adv. in Space Res., in press 1993

Targets

	Projektil	Helium	Neon	Argon
1	5.9 MeV/u Ne^{7+}	30–140°	30,50,120°	30–140°
2	5.9 MeV/u Ar^{10+}	30–140°	30–140°	30–140°
3	5.7 MeV/u Ar^{18+}	30–140°	30–140°	30–140°
4	3.5 MeV/u Ni^{22+}	30°	—	27–60°
5	5.4 MeV/u Kr^{31+}	30–50,110°	30–140°	—
6	2.4 MeV/u Xe^{21+}	17.5–70°	—	25–45°
	3.6 MeV/u Xe^{21+}	30–90°	30–90°	30–90°
	6.0 MeV/u Xe^{21+}	30–140°	30–140°	30–140°
7	6.0 MeV/u Au^{24+}	32–145°	30–145°	30°
8	1.4 MeV/u Bi^{26+}	30–80°	—	30°
	2.9 MeV/u Bi^{26+}	30–90°	—	—
	3.6 MeV/u Bi^{26+}	30–90°	—	—
	6.0 MeV/u Bi^{26+}	27–90°	—	—
9	5.9 MeV/u Pb^{26+}	30°	30°	30°

Progress Report

Contract: FI3P-CT920007 Sector: B12

Title: Molecular basis of radiosensitivty.

1)	Lohman	Univ. Leiden
2)	Bridges	MRC
3)	Bootsma	Univ. Rotterdam - Erasmus
4)	Moustacchi	CIR
5)	Thacker	MRC
6)	Backendorf	Univ. Leiden
7)	Eckardt-Schupp	GSF

I. Summary of Project Global Objectives and Achievements

(i) Identification of radiosensitive individuals both in the normal population and in individuals with known radiosensitive genetic disorders.
(ii) Understanding of the cellular response to radiation.

(i) The single cell microgel (comet) assay has been successfully employed to measure DNA strand breakage in human T-lymphocytes, and to demonstrate the role of radical scavenges in radiation induced strand breakage. The latter has been achieved by modulation of dietary levels of vitamin C in a panel of normal volunteers. Radiation induced cytotoxicity and micronuclei have been assayed to investigate potentially lethal and clastogenic damage in breast cancer patients and to identify radiosensitive individuals in the normal population as well as ataxia-telangiectasia heterozygotes. Two unique radiosensitive individuals have been identified. The data obtained in the micronucleus assay are in a format requiring detailed statistical analysis, but it is clear that AT heterozygotes can be distinguished. In Fanconi anemia (FA) cells an unbalanced cytokine production was demonstrated. In particular abnomalies in tumor necrosis factor α (TNFα) appear to be a general characteristic associated with FA and is a possible tool in detecting FA heterozygous individuals. The abnormal high production of TNFα is also seen in AT cell lines. A systematic study to test TNFα concentrations in AT families is organized.

(ii) Different and complementary strategies have been used in the different laboratories to study cellular responses to radiation.

Cloning of repair genes. The human gene correcting the radiosensitive XRS mutants is located on a small subfragment of chromosome 2, closely linked to the TNP-1 marker. Cloning of the human DNA has been started and several positive YACs have been obtained. The human gene complementing the irs1 mutation and involved in resis-

tance to ionizing radiation, is probably located on chromosome 7. A hybrid cell line, made by fusing of human cells and hamster irs1 mutant cells, was found to be radiation resistant and carried an additional chromosome part of which was derived from human chromosome 7. Human sequences from the hybrid line are amplified and used to screen a chromosome 7 specific YAC library.
Isolated yeast genes have been used to clone homologous human genes. In this way two human RAD6 homologs and the human Rad23 gene were cloned. Progress has been made in cloning S. pombe radiosensitivity genes involved in excision repair and cell cycle progression. Two new highly conserved genes involved in excision repair and several genes involved in G2 arrest following irradiation, have been cloned and sequenced.

Functional analysis of encoded proteins and identification of proteins in cell free in vitro systems. The cloned human repair genes ERCC1, XPBC/ERCC3 and CSBC/ERCC6 have been further characterized with regard to functional domains and interaction with other proteins. The presence of an enzyme complex harbouring the products of ERCC1, ERCC4, ERCC11 and XPFC genes was demonstrated by an in vitro assay. It is postulated, that this complex functions both in nucleotide excision repair and recombinational repair.
Mutations in ERCC3 are responsible for xeroderma pigmentosum group B. Spectacular results have been obtained by the demonstration that the ERCC3 protein is part of a multiprotein complex (TF11H) required for transcription initiation and excision repair. Part of the clinical symptoms of the corresponding disorder can now be explained as the result of a subtle impairment of transcription.
Transfection experiments revealed that the ERCC6 gene specifically corrects the repair defect in cells of Cockayne's syndrome complementation group B shown to be defective in preferential repair of transcriptionally active genes.
Using the recently developed technology of gene targeting in mouse stem cells (ES-cells) one of the allele of ERCC1 and 3 was inactivated. Several ES recombinants were obtained and injected in mouse blastocysts. The eventual aim is to generate mouse mutants with the same repair defect as in human syndroms.
In vitro assays using plasmid DNA and cell-free extracts have been employed to identify proteins involved in the repair of DNA double-strand breaks induced by restriction enzymes (DSBs). Four separate break rejoining activities have been isolated and are further characterized. One of this fractions contains DNA ligase I as the only detectable ligase. In addition the in vitro approach can be used to study the misrepair of DNA strand breaks. Sequence analysis of misrejoined DNA demonstrated that certain short direct repeat sequences are involved in the process. In extracts from AT cell lines the misrejoining frequency is increased by 10-fold or more.

Topography of DNA repair. To investigate a possible influence of DNA sequence and chromatin structure or induction and repair of radiation induced DNA damage, pulse field gel electrophoresis and computer analysis of DNA profiles have been employed. The distribution of DNA double strand breaks in the yeast genome induced by ionizing radiation under hypoxic or oxic conditions, is similar and random. The methodology is under development for analysis of radiation induced DNA damage in mammalian cells.
Induction and repair of UV-induced pyrimidine 6-4 pyrimidone photoproducts (6-4 PP) have been studied at the gene level in normal, xeroderma pigmentosum and Cockayne's syndrome fibroblasts. No distinct differences in induction frequencies were found between active and inactive genes. Repair of 6-4 PP is faster in active genes during the initial phase following UV-irradiation, but preferential repair of the transcribed strand was absent. CS cells showed a reduced rate of repair of 6-4 PP of active genes.
Permeabilized cells have been employed to study the role of chromatin structure in UV-induced repair by in vitro reactions. A substantial part of total cellular repair capacity retains with chromatin even after extensive extraction, suggesting an association of repair enzymes with a nuclear skeleton i.e. the nuclear matrix. Repair activity is not retained with chromatin in CS cells defective in repair of active genes.

Mutagenesis. The major types of mutations induced by ionizing radiations are large deletions and rearrangements. Analysis of several X-ray induced mutations in the HPRT gene indicate that many mutants have lost the whole gene and mapping of breakpoints requires molecular probes outside the gene. Anonymous sequences mapped around the HPRT gene region Xq26 are used to probe for absence/presence of defined sites in mutants. In one complex mutant a breakpoint within one flanking site has been identified. Breakpoints at deletions induced by α-particles, have been mapped internally to the gene; in one case short direct repeats were shown to be involved in the formation of the deletion.
In radioadapted lymphoblastoid cells less deletion type HPRT mutants are observed, possible due to a triggering of double strand break repair in adapted cells. UV-induced mutations at the HPRT gene have been analysed in normal and XP-C lymphoblastoid cells. No clear correlation was found between repair phenotype and distribution of mutation as previous shown for hamster cells and it is now obvious that UV-induced mutation spectra are quite different for exponentially growing human and hamster cells. Sequence analysis at the deletion breakpoints in spontaneous HPRT mutants in Fanconi anemia (FA) cells, revealed features typical for V (D) recombinase-mediated cleavage and joining. This suggests that illegitimate recombination may become active in FA pathology.

Effects of radiation on cell proliferation and differentiation. The expression of a novel class of genes (SPRR genes) tightly regulated during the maturation of epidermal cells, is disturbed by UV- and ionizing radiation. The expression of SPRR2 genes is subjected to both positively and negatively acting regulatory factors (transcription factors). One of the development regulators (the POU domain protein OCT-1) interferes with the SPRR2 promotor. However, no change in binding of the OCT-1 was observed after radiation, and no clear evidence has obtained for a direct involvement of this protein in regulation of SPRR genes. The expression of SPRR2 genes is inhibited by the AP-1 family of transcription factors: a decrease of AP-1 binding activity is concomitant with an increase of SPRR2 expression. The following model is proposed: In proliferating basal cells the AP-1 activity is high and the SPRR2 gene is repressed. When basal cells differentiate, AP-1 activity drops and SPRR2 will be expressed. UV-irradiation of basal cells results in a decrease of SPRR2 expression, and an increase in AP-1 activity. This response is specific for UV-irradiation.

Head of project 1: Prof. Dr. Lohman

II. Objectives for the reporting period

(i). Analysis of the role of chromatin structure in the induction and repair of DNA damage in UV-exposed mammalian cells.
(ii). Analysis of DNA repair in vitro using intact chromatin as substrate.
(iii). Investigation of the mutagenic consequences of different repair phenotypes by molecular analysis of UV-induced mutation spectra in normal and UV-sensitive mammalian cells.
(iv). Isolation and characterization of mammalian cell lines sensitive to UV-light and ionizing radiation.
(v). Cloning of mammalian DNA repair genes using repair deficient hamster cells or cloned yeast genes as heterologous probes.

III. Progress achieved including publications

(i) Chromatin structure and repair.
Analysis of repair at the gene level has revealed distinct differences in the rate and extent of repair of UV-induced cyclobutane dimers (CPD) between transcriptionally active and inactive sequences. The CPD repair phenotypes observed in normal and UV-sensitive human cells (xeroderma pigmentosum, Cockayne's syndrome), suggest various hierarchies of DNA repair: (i) a slow repair of inactive genes, (ii) the fast repair of active genes, and (iii) the accelerated rate of repair of the transcribed strand of active genes (transcription coupled repair). Analysis of frequencies of CPD in the skin of hairless mice exposed to UV-B light has revealed that such hierarchies exist in vitro as well.
We have developed the methodology to determine the frequency of another important UV-induced photolesion in specific DNA sequences i.e.the pyrimidine 6-4 pyrimidone photoproduct(6-4PP).The method is based on sequential incubation of purified DNA obtained from UV-irradiated cells with photolyase and E.coli UvrABC excinuclease complex. With this technique we measured an induction of one 6-4PP per 4 CPD both in active and inactive sequences.In normal human fibroblasts 6-4 PP are extremely fast repaired from both active and inactive se-

quences, although the rate of repair in active genes is higher during the initial phase following UV-irradiation. No strand - specific repair of 6-4PP is detected in normal cells. These results correlate well with immunological measurements of BUdR labelled repair sites which also show the absence of strand specific and preferential repair. These results indicate that transcription coupled repair of 6-4 PP is too slow to compete with an efficient global repair system.

(ii) In vitro repair of UV-induced damage.
We have used cells encapsulated in agarose beads,to measure DNA repair in intact chromatin by in vitro reactions. Cells are lysed and extracted with buffers that mimic the physiological conditions of the cell as much as possible. Incorporation of radioactive dNTPs is stimulated in UV-irradiated confluent normal fibroblasts and depending on Mg^{2+} and ATP. No such incorporation was found when cells were lysed prior to UV-irradiation suggesting that the cells have to be primed for repair in vivo. About 20% of the total repair activity in permeabilized cells is tightly associated with the chromatin in normal and XP variant cells, but absent in XP-C and CS cells. These results suggest that analogous to replication and transcription, repair is compartmentalized within the nucleus.

(iii) Mutagenesis
Mutations in the HPRT gene have been analyzed in normal and XP-C lymphoblastoid cells. In XP-C cells CPD are selectively removed from the transcribed strand of the HPRT gene, whereas both strands are repaired in normal cells. The expectation that most of the mutations in XP-C cells would be derived from lesions in the poorly repaired non-transcribed strand of the HPRT gene, was not fulfilled. Instead mutations in XP-C cells are generated equally from both strands. This is possible due to the relative slow but strandspecific repair of 6-4PP in these cells. Consequently, 6-4PP at the transcribed strand (known to be very mutagenic) will contribute significantly to the induced mutations.
The molecular nature of UV-induced HPRT mutations in UV-sensitive Chinese hamster ovary cells mutant UV24 indicate that under defective nucleotide excision repair conditions the induction of mutations is strongly biased towards lesions in the transcribed strand of the HPRT gene. A plausible explanation for this is that during replication large differences exist in the error rate with which DNA polymerases bypass lesions in the templates for the leading and lagging strand, respectively.

(iv) Isolation and characterization of mammalian cell lines sensitive to UV light and ionizing radiation
Seven new X-ray-sensitive mutants have been isolated (six from Chinese hamster V79B cells and one from BHK cells). These new mutants are being characterized. A new mutant (XR-V9B) defective in DNA double-strand break repair was described. This mutant is also affected in V(D)J recombination indicating that double-strand break repair in XR-V9B and V(D)J recombination share the same factor. The radiosensitive AT-like Chinese hamster cell mutants isolated previously, were further characterized. The only slightly increased X-ray-induced mutability

in these mutants, indicates that the lesions which are not repaired affect mainly chromosomal instability. Despite the fact that they mimic the phenotypic characteristics observed in cultured cells from A-T patients the mutants are not homologous to AT-A, AT-C nor AT-D, as the genes are not localized on human chromosome 11. Using microcell-mediated chromosome transfer several human chromosomes were eliminated as the candidates for the gene localization on the human genome.
The relation of radiosensitivity and radioresistant DNA synthesis (RDS) was studied in the AT-like hamster cell mutants. RDS behaves as a (co-) dominant feature in hybrids of AT-like mutants and wild-type cells, indicating that RDS cannot be used for complementation analysis of X-ray-sensitive rodent cell mutants. In contrast to the intraspecies hybrids, a full complementation of RDS was observed in interspecies hybrids between the hamster AT-like mutants and human HeLa cells, suggesting a species-specific regulation of DNA synthesis.
A Chinese hamster ovary cell mutant EM-C11 defective in the XRCC1 gene was isolated and described. The results suggest that this gene is mainly involved in repair of DNA damage induced by alkylating agents.

(v) Cloning of mammalian DNA repair genes.
To clone a human gene complementing the defect in the AT-like mutants, the V-C4 mutant was used as a recipient for genomic transfection of normal human DNA. Sofar, one primary transformant with an intermediate X-ray-resistance was isolated. When a cosmid library of normal human DNA was used one primary transformant (PTcos-1) also with the intermediate X-ray-resistance was obtained. This intermediate X-ray resistance suggests that the defective human gene is not fully homologous to the rodent counterpart. Presently, the primary transformant (PTcos-1) is being used to rescue the complementing human sequences.
Repair deficient mutants of the yeast *Saccharomyces cerevisiae* belonging to the RAD52 epistasis group, RAD50-57, are involved in the recombinational repair of double strand breaks in DNA. Among this group, mutations in RAD51, RAD52 and RAD54 confer the most extreme senstivity to ionizing radiation and MMS. To study the evolutionary conservation of the RAD52 group genes we isolated the RAD51 and RAD54 homologs from the distantly related yeast strain *S.pombe*. Sequence analysis showed a remarkable similarity at the amino acid level of ScRAD51 and SpRAD51 (69%) and of ScRAD54 and SpRAD54 (56%). A *S.pombe* strain containing a disrupted RAD51 gene is also very sensitive to X-rays. Together, these results indicate evolutionary conservation of the recombinational repair pathway. The sequence homology between related genes from both yeast strains, can be used to design degenerate primers in order to amplify homologous genes from other organisms. In this way the RAD52 gene from mouse has been isolated recently.

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Head of project 2: Prof. Bridges

II. Objectives for the reporting period

Identification of radiosensitive individuals

Measurement of radiation sensitivity of fibroblasts and lymphocytes from normal individuals, breast cancer patients and ataxia-telangiectasia heterozygotes using survival, micronucleus induction and single cell microgel assay as endpoints.

Cloning of human DNA repair genes

a) Working towards the cloning of the human *xrs* gene.
b) Using *S. pombe* radiosensitivity genes as models for human genes and to clone the latter using PCR methods.

Objectives for the next reporting period

Identification of radiosensitive individuals

a) Analysis of completed experimental work on breast cancer patients and ataxia-telangiectasia heterozygotes.
b) Use of single cell microgel assay to assist understanding of genetic and non-genetic components of radiosensitivity.

Cloning of DNA repair genes

a) Introduction of candidate YAC clones into *xrs* mutants by protoplast fusion; analysis for correction of repair defect.
b) Complementation of *S. pombe* mutants by human DNA library in *S. pombe* expression vector to obtain functionally homologous human genes. Further study of novel genes involved in excision repair and of the arrest of cell cycle progression following radiation.

III Progress achieved including publications

Identification of radiosensitive individuals

a) Cell Survival. We have continued to add to our database for survival of fibroblasts and T lymphocytes from normal individuals and have largely completed an analysis of the efficiency of the repair of potentially lethal damage in normal and radiosensitive individuals. Certain syndromes such as Gorlin's syndrome prove to be radiosensitive under protocols which exploit differences in this mode of repair.
Two unique radiosensitive individuals have been identified. The first (290BR), reminiscent of a patient whose cells we have already reported on, is clearly distinct from ataxia-telangiectasia (A-T) and may represent a new entity. The

second (XP14BR), is a xeroderma pigmentosum donor from complementation group C and represents the second instance of the combination of ultraviolet and ionising radiation sensitivity individuals with this disorder.

b) The Micronucleus Assay. A substantial study has been completed of the repair of radiation induced clastogenic damage (as micronuclei) in fibroblasts from normal, A-T patients, A-T heterozygotes and a panel of breast cancer ptients. The data are in a format requiring detailed statistical analysis which will be progressed as an objective for the next reporting period. It is clear however that A-T and A-T heterozygotes can be distinguished from normals in this series of experiments.

c) The Single Cell Microgel (Comet) Assay. We have shown that this assay is an efficient *ex vivo* method for measuring strand breakage in human T-lymphocytes. The involvement of radical scavengers in the expression of radiation induced strand breakage has been demonstrated following the modulation of dietary levels of vitamin C in a panel of normal volunteers.

Cloning of DNA repair genes

a) *xrs* gene. We have shown previously that the human gene correcting the radiosensitive hamster *xrs* mutants is located on chromosome 2. Using radiation reduction we have obtained a radioresistant hybrid, in which the only human material was a small subfragment of chromosome 2 located at 2q35. This hybrid contains the TNP-1 marker and the *xrs* gene, but all other chromosome 2 markers that we have tested are absent, indicating close linkage between the *xrs* gene and TNP-1. We have begun to clone the human DNA present in this cell line using Alu-PCR and YAC screening. Alu-PCR produces a series of products which have been cloned into a "T-vector" and used as probes against gridded YAC libraries. Several positive YACs have been obtained and are currently being analysed. We have also produced a physical map of the DNA surrounding the TNP-1 marker using pulse-field gel electrophoresis on four TNP-1-containing YACs which we have received from elsewhere.

b) *S. pombe* radiosensitivity genes.

(1) Excision repair. We have cloned and analysed two new genes involved in excision repair of DNA damage. *S. pombe rad2* is a member of the family of genes including human ERCC5/*S. cerevisiae RAD2*/*S. pombe rad13*. The product of *S. pombe rad18* is an alpha-helical leucine-zipper protein with an ATP-binding site. We have identified *S. cerevisiae* homologues of both these genes, demonstrating that these genes are highly conserved.

(2) Cell cycle progression. We previously showed that four radiation-sensitive mutants *rads 1, 3, 9, 17* failed to undergo G2 arrest following irradiation. We have isolated several more mutants with similar phenotypes (designated *rad24-28*) and we have cloned the genes defective in these mutants. Analysis of the sequences of encoded gene products suggests that *rad27* is a protein dinase, *rad25* is a protein kinase inhibitor and *rad28* is a ubiquitin conjugating protein.

rads 1, 3, 9, 17 and *26* fail to arrest cell cycle progression following irradiation and also following inhibition of DNA synthesis by hydroxyurea. In contrast *rad27* fails to arrest after irradiation, but does arrest after hydroxyurea treatment. Furthermore whereas arrest following hydroxyurea has been shown to require the products of the *cdc25* and *wee1* genes, we have conclusively demonstrated that arrest after irradiation is similar in normal and *wee1* cells. These data show definitively that although the arrest of cell cycle progression following irradiation and hydroxyurea treatment involves many of the same gene products, the mechanisms by which arrest is effected are quite different.

(3) construction of a human cDNA library in a *S. pombe* expression vector is close to completion.

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Head of project 3: Prof. Dr. D. Bootsma

II. Objectives for the reporting period

(i) Cloning and characterization of human repair genes via genomic or cDNA transfection to repair deficient rodent or human mutants or by sequence homology to cloned yeast repair genes. (ii) Functional analysis of the encoded proteins, involving generation of monospecific antibodies, purification of the (overexpressed) proteins, assay for activity using a cell-free *in vitro* excision repair system or microneedle injection into repair-deficient XP cells, analysis of specific functions (such as: lesion-specific DNA binding and incision, DNA unwinding or other DNA metabolizing activities) and interaction with other polypeptides of the DNA repair, recombination or replication machinery.
(iii) Generation of a mouse model for XP/CS and other repair deficiencies based on recently developed techniques for targetted gene replacement in totipotent embryo-derived stem cells (EC-cells) from which mouse germline chimera's and eventually homozygous mouse mutants can be derived. Such repair-deficient mouse strains will be invaluable for assessing the biological role of repair and other factors in carcinogen-induced tumorigenesis, as an experimental model for XP and CS (etiology, treatment and prevention of the disease), for the sensitive screening of radiation and chemical agents for their carcinogenic, mutagenic and cytotoxic potential *in vivo*, and for risk assessment of heterozygotes, carrying a recessive repair defect.

III. Progress achieved including publications

To investigate the mechanism of NER and to resolve the molecular defect in xeroderma pigmentosum (XP) and Cockayne Syndrome (CS) we have focused on the cloning of human DNA repair genes. Two strategies were followed for the isolation of these genes:

a. DNA transfection to repair-deficient rodent (at least 10 different complementation groups) and human cells followed by selection of UV-resistant transformants and 'rescue' of the correcting gene. This has resulted in the cloning of *ERCC1, XPBC/ERCC3 and CSBC/ERCC6.*
b. Sequence homology to cloned repair genes of lower eukaryotes, particularly yeast (*S.cerevisiae*). This approach has yielded *HHR6A*, *HHR6B* and *HHR23.*

The *ERCC1* gene corrects the repair defect of rodent group 1 mutants and encodes a 297 amino acid (aa) protein. For the first 214 residues *ERCC1* displays overall homology to the yeast NER protein RAD10 (210 aa) and in the remaining part local homology to segments of the *E.coli* NER proteins UvrA and UvrC. Mutation analysis suggests that *ERCC1* has distinct roles in the repair of UV-lesions and in the processing of cross-links. By using an *in vitro* repair system we demonstrated the presence of an enzyme complex in mammalian cell extracts, harbouring the products of the *ERCC1, ERCC4, ERCC11* and *XPFC* genes. We postulate that this complex, like the one in *S.cerevisiae* involving the RAD1 and RAD10 proteins, functions both in NER and recombinational repair, and may play a role in strand incision.
The *ERCC3* gene corrects UV-sensitive rodent mutants of group 3. It specifies a protein of 782 amino acids. The deduced amino acid sequence suggests it to be a chromatin binding helicase. No homologous (repair) proteins in any organism were identified in data bases. However, the gene appeared very strongly conserved, which permitted isolation of the *Drosophila* and yeast (*S.cerevisiae* and *S.pombe*) homologs. Gene disruption in yeast indicated that ERCC3^{sc} participates in a process essential for viability in addition to its

role in NER. Transfection and microinjection experiments demonstrated that mutations in *ERCC3* are responsible for xeroderma pigmentosum (XP) complementation group B, a very rare form of XP that is simultaneously associated with Cockayne's syndrome (CS). Using microinjection of the gene, 2 new patients were assigned to this group. In spite of the severe NER deficiency, both patients have not developed skin cancer (in striking contrast to the sole known XP-B case) even at relative advanced age. This points to the involvement of additional factors required for cancer proneness.
In collaboration with the laboratory of J-M. Egly (Strasbourg) the ERCC3 protein was found to be part of a multiprotein complex (TFIIH) required for transcription initiation of most structural genes and for NER. This defines the additional, hitherto unknown vital function of the gene, suspected from parallels with the yeast and Drosophila *ERCC3* homologs. Importantly, the complex in fact appeared to contain at least 4 proteins implicated in different XP/CS complementation groups. Part of the clinical symptoms of the corresponding disorders that were difficult to interpret on the basis of a DNA repair defect can now be fully explained as the result of a subtle impairment of transcription.
The *ERCC6* gene, was cloned after extensive genomic DNA transfection to a member of the moderately UV-sensitive rodent group 6. The -for transfection cloning- very large gene ($\geq$ 85 kb) encodes a protein of 1493 amino acids. *ERCC6* is after *ERCC2* and *ERCC3* the third DNA helicase in mammalian NER. No equivalent of *ERCC6* is known in any species. Transfection experiments revealed that the gene specifically corrects the repair defect in cells of CS complementation group B shown to be impaired in the preferential repair of (the transcribed strand of) active genes (Troelstra *et al.*, 1992). The *CSBC/ERCC6* gene is the first cloned gene specifically implicated in this subpathway of NER.
The strong evolutionary conservation of most of the repair genes analyzed until now has prompted us to see whether isolated yeast genes could be recruited to identify and clone homologous human genes. This approach was applied to the *S.cerevisiae RAD6* gene, which plays a central role in the postreplication repair, damage-induced mutagenesis and sporulation. The 172 amino acids RAD6 protein is a ubiquitin-conjugating enzyme (E2) that *in vitro* (poly)ubiquitinates histones. By evolutionary walking based on nucleotide sequence homology we have cloned two human *RAD6* homologs (designated *HHR6A* and *HHR6B*) using the *Schizosaccharomyces pombe* and *Drosophila melanogaster* genes as "intermediates". *HHR6A* and *B* probably function in chromatin remodelling. Immuno-electronmicroscopy suggests that both proteins are associated with euchromatin.
We also cloned the human homolog of the yeast *NER* gene *RAD23*. Sequence analysis indicated that the ubiquitin-like N-terminus as well as the remainder of the protein are clearly conserved. Characterization of this gene, that in yeast has only a partial involvement in NER, is in progress.
Using the recently developed technology of gene targeting in totipotent embryo derived mouse stem cells (ES-cells) we have inactivated one of the alleles of *ERCC1* and *3*. Because of the possible vital role of both genes also constructs were made which induce more subtle mutations and -in the case of *XPBC/ERCC3*- mimick one of the mutations found in an XP-B patient. For each gene and type of mutation several homologous ES recombinants were obtained and injected into mouse blastocysts. After implantation into pseudo-pregnant foster mothers a number of *ERCC1* and *ERCC3* chimeric mice were born, that are now being tested for germline chimerism. The eventual aim is to generate by interbreeding mouse mutants with the same biochemical defect as in human repair syndromes and patients. Such experimental mouse models are extremely valuable for studying the relationship between repair deficiency and cancer, risk assessment, genotoxicity and carcinogenicity testing etc.

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Head of project 4: Dr. Moustacchi

II. Objectives for the reporting period

In order to understand the mechanisms of human DNA repair processes and to elucidate the molecular basis of induced mutations in mammalian cells including man, we have focused on :

1. The development of various experimental means for detecting individuals abnormally sensitive to genotoxic agents including ionizing radiations.

2. The molecular analysis of induced mutation in human lymphocytes and lymphoblasts.

III. Progress achieved including publications

Sub-project 1

The single cell gel electrophoresis assay also denominated the "comet assay" is a rapid, simple and sensitive technique for analysing DNA strand breaks induction and repair in human cells. After a stay of one of the ICP collaborators at the CMU, the procedure has been established at the ICP using the automated Colourmorph Interactive Image Analysis System. The aim is to apply this test on a relatively large scale basis to breast cancer patients treated at the Curie Hospital and analysed in parallel for their mutagenic response at the *HPRT* locus in lymphocytes (Sala-Trepat et al., 1990). As it has been strongly suggested that about 10% of such patients are heterozygous for the ataxia telangiectasia (AT) gene, it is of interest to determine if, according to such two parameters (strand breaks and their repair and mutation induction), AT heterozygotes are detectable. The comet assay has been applied using conventional experimental protocols to human lymphocytes, lymphoblasts and fibroblasts following ^{137}Cs γ-irradiation at various fluences. The repair kinetics for normal and AT donors have been established and our data are in good agreement with those previously obtained by Green et al. at the CMU. We are now attempting to apply the comet assay to mammary epithelial cells derived from breast cancer cytopunctions (a mean of 10^5 cells) performed before the start of any treatment and following the clinical and anatomo-pathological diagnosis. Preliminary experiments indicate that such a cell type demonstrates less heterogeneity in response to γ-rays than lymphocytes or lymphoblasts from the same patient. It is also planned to apply the test to biopsy specimens.

We have demonstrated an unbalanced cytokine production in Fanconi anemia (FA) cells, i.e. an underproduction of interleukin 6 (IL6) during growth (Ref. 1). Among a number of cytokines analysed, the only other presenting anomalies was tumor necrosis factor α (TNFα). In comparison to normal cells, this cytokine is overproduced by FA lymphoblasts derived from the 4 genetic complementation groups (Ref. 17). Moreover, addition of anti-TNFα antibodies partially corrects the FA hypersensitivity to treatment by mitomycin C (MMC). No anomalies at the molecular level (Southern and Northern blot analysis) were detected for the TNFα gene and its mRNA. The three AT cell lines analysed demonstrated a normal production of IL6 but, shared with FA, an abnormally high production of TNFα. In contrast to normal healthy donors or to aplastic anemia patients, in which serum TNFα is present only in trace amount, 36 FA individuals and 21 FA parents (heterozygotes) monitored show a significantly ($P < 0.001$) higher level of serum TNFα activity. Consequently, abnormal TNFα production appears to be a general phenotypic characteristic associated with the FA genetic background (Ref. 17). We suggest that the observed anomalies in such cytokine production may contribute to the complex phenotype of the FA syndrome which includes defective differenciation of the hematopoietic system, chromosomal instability and a predisposition to cancer. Moreover, although a larger group of donors of different geographic and ethnic origin should be examined in the future, our results suggest that the serum level of TNFα may be utilized as a possible end point in detecting heterozygote carriers of the FA gene(s) within families with an affected child. A similar study on AT families is organized (collection of serum samples) and will be started.

Sub-project 2

We have reported a 70% reduction in *HPRT*$^-$ mutant frequency in radioadapted (2 cGy followed after 6 hrs by 2 Gy to 4 Gy) human lymphoblastoid cells (Ref. 7). A collection of 118 mutants has been analysed for structural rearrangements of the *HPRT* gene using the multiplex PCR assay and the Southern blot method. The overall results show that the deletion type mutants represent 77% of the single high dose (double strand breaks or DSB) whereas only 42% of such type of mutants are found in radioadapted cells (repair of DSB is probably triggered). Mutants with no change at the *HPRT* gene level were characterized for the gene expression. The vast majority of adapted mutants (86%) were still expressing mRNA whereas *HPRT* transcripts were detected in only 56% of the mutants induced by the high dose alone (aberrant splicing).

An increased frequency of gene deletion is an important cellular feature of Fanconi anemia (FA) (Papadopoulo et al., 1990 and Ref. 12-13), an inherited DNA repair disorder which shares with AT a predisposition to leukemia and a chromosomal instability. Sequence analysis at the breakpoint junctions in deletions formed at the *HPRT* locus revealed several features typical for V(D)J recombinase-mediated cleavage and joining (Ref. 19). This strongly suggests that this illegitimate recombinational activity, which is switched off in normal cells, becomes activated in the FA pathology. Anomalies in recombinational activity have also been recently reported for AT (Meyn, 1993).

Finally, a genomic DNA fragment has been cloned from FA cells, group D (previously denominated "B"), corrected by mouse genomic DNA for their mitomycin C hypersensitivity. This fragment maps to chromosome 11q23 (Ref. 20), a region which contains several hot spots of chromosomal instability associated with solid tumors and a gene likely to determine differenciation of primitive hematopoietic stem cells according to Rowley et al. (1990). A human cDNA has been isolated. The gene (98 kDa) corresponds to a "motor protein" and bears GTPase sequence homology. This FA(D) candidate gene is now being analysed for presence of mutations in FA(D) cells DNA.

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Head of project 5: Dr. Thacker

II. Objectives for the reporting period

To assess the feasibility of using a complementing human/hamster hybrid line to map the chromosomal position of the human equivalent of the *irs1* radiosensitivity gene. To establish robust cell-free methods for the molecular analysis of rejoining of DNA double-strand breaks, using substrates with either homologous or non-homologous termini. Cell extracts will be fractionated and components with break-rejoining activity identified. Mis-rejoining at specific break-sites will be analysed by engineering sites and by reconstruction of the process using purified enzymes. We will induce and isolate large numbers of mutants by α-particles in primary human cells, to complement our existing X-ray set. Using PCR-based methods, we will analyse the sites of mutation and attempt to sequence breakpoints of large deletions and rearrangements, as well as mapping those breakpoints that occur outside the gene.

III. Objectives for the next period

To exploit PCR-based methods and radiation-reduced hybrids to closely map the site of the human gene complementing the radiosensitive *irs1* mutant. A comprehensive YAC library for chromosome 7, available through collaboration, will be used to attempt the cloning of this repair gene. The purification of proteins involved in correct rejoining of both homologous and non-homologous broken DNA ends will be pursued. New substrates will be designed to optimize mis-rejoining of broken DNA, to examine in molecular detail the formation of deletions. The spectrum of mutations induced by α-particles in primary human cells will be assessed, and compared to our data for X-rays. Mapping and sequencing of breakpoints of large deletions in genomic DNA will be continued to establish mechanisms of mutation induced by high and low LET radiations.

IV. Progress achieved including publications

1. Mapping/cloning of the human gene complementing the irs1 *mutation.* The mapping and cloning of genes that are involved in resistance to ionising radiation damage remains a most important aim of the programme. The *irs1* line, derived in our laboratory from V79-4 hamster cells, is hypersensitive to ionising radiations, as well as being extremely sensitive to DNA cross-linking agents such as mitomycin-C. A hybrid cell line, made by fusing human cells with *irs1*, was found to be resistant to both radiation and mitomycin-C suggesting that it carries the human gene complementing the *irs1* mutation (kindly supplied by Dr. L. Thompson, Lawrence Livermore National Laboratory, USA). This hybrid line was characterized for its human chromosomal content, using fluorescence *in situ* hybridization (FISH). The hybrid was found to contain a small additional chromosome, part of which was derived from human chromosome (chr.) 7. Using interspersed repetitive PCR (single primers complementary to either end of the human *alu* repeat), a large number of human sequences were amplified from this hybrid and cloned into T-vectors. These cloned sequences were screened for internal repeats and for chr.7 complementarity. The non-repeat sequences derived from chr.7 are then being used to screen a chr.7 YAC library (in collaboration with Drs. S. Scherer and L-C. Tsui, Hospital for Sick Children, Toronto). Additionally Southern hybridization (with genomic probes mapped to chr.7) and single-probe FISH have identified the chr.7 region present in the hybrid.

2. Molecular analysis of repair/misrepair of site-specific DNA breaks.

We have developed *in vitro* systems for the identification of proteins involved in the correct repair of DNA double-strand breaks (dsb), the major lesion induced by ionising radiation. The assay utilizes cell-free extracts to repair dsb with defined termini produced by restriction enzymes. The rejoining of homologous termini has been studied in detail and extracts from either cultured human cells or calf thymus have been separated into several active components. Four biochemically separate break-joining activities have been identified and are being further characterized. One of these is active only in the presence of a stimulating factor (REP1, see below) and we have shown that this fraction contains DNA ligase I as the only detectable ligase. Conversely, another fraction has been found to be devoid of ligase I by adenylation and Western analysis, but still has rejoining activity. We previously identified a rejoin-enhancement protein, REP1, and this has now been extensively purified and its biochemical properties such as co-factor dependence and binding properties have been examined. REP1 is of very low abundance and we are at present trying to accumulate large amounts of it to complete the purification to homogeneity.

An analogous system for the rejoining of non-homologous termini is also being developed, since this may relate more closely to the type of terminus found at radiation-induced dsb. The optimization of *in vitro* conditions has been assessed using a number of different mis-matched termini.

In addition we have examined the misrepair of DNA breaks, using a similar *in vitro* approach. The frequency of mis-rejoining is dependent on both the initial break-site and the cell-line extract used. For example, mis-rejoining occurs at about 0.5% for breaks treated with extracts from 'normal' human cells at an *Eco*RI site in the pUC18 molecule. However, with extracts from two cell lines derived from patients with the radiosensitive disorder ataxia-telangiectasia (A-T), the mis-rejoin frequency is increased by 10-fold or more. After sequencing a large number (>50) of mis-rejoined molecules, it was found that certain short direct repeat sequences were involved in the process (i.e., deletion formation around the initial break-site). One 'hotspot' for deletion formation was eliminated by engineering the DNA molecule, without affecting the initial break-site, and the frequency of mis-rejoining by A-T cell extracts was then found to return to normal. This proved that mis-rejoining does not simply depend on the initial dsb but also on secondary effects relating to flanking DNA sequence.

An attempt was also made to 'reconstruct' the mis-rejoining process using purified prokaryotic enzymes. Defined templates carrying a site-specific dsb were treated initially with different exonucleases to give single-strand degradation, followed by addition of DNA ligase and polymerase. While high levels of mis-rejoining were induced under these conditions, sequencing of the molecules showed that the mechanism was not the same as that found with human extracts (i.e., it did not involve short direct repeats). Thus, the direct repeats do not simply come together in transient interactions which are fixed by polymerase and ligase action; additional factors must be involved when the mis-rejoin process is driven by human extracts.

In a separate approach we have electroporated restriction enzymes into human cells of differing radiosensitivity, to see if these are similarly sensitive to site-specific dsb. Using immortalized lines of cells from Bloom's syndrome, DNA ligase I deficiency (line 46BR), ataxia-telangiectasia, and from a normal individual, a linear correlation was found between sensitivity to radiation and to a restriction enzyme giving blunt-ended breaks (but not to an enzyme giving ends with homologous base-overlap).

An attempt was also made to extend the *in vitro* work on mis-rejoining (deletion formation) to cellular mutation response. Using an integrated target gene to facilitate rapid molecular analysis, restriction enzymes yielding different break-end structures were electroporated into cells. It was found that a few enzymes did induce high frequencies of mutation provided selection was not stringent. However, this effect did not show any apparent dependence on break-end structure. The use of isoschizomers and the development of methods for the determination of enzyme activity under simulated cellular conditions, showed that most enzymes were ineffective because the enzyme was inactive in intracellular conditions. Interestingly, however, a low mutant frequency (and very low survival) was also found with certain enzymes having very long half-lives under simulated cellular conditions, suggesting that constant acute levels of dsb damage can lead exclusively to lethality.

3. Molecular analysis of radiation-induced mutations in primary human cells.

We have established from previous work that the major types of mutation induced by ionising radiations are large deletions and rearrangements. While these mutations are difficult to study at the molecular level, due to their large size, we have previously succeeded in the isolation and analysis of several X-ray induced mutations in the *HPRT* gene of primary human fibroblasts. Many of the radiation-induced mutants, however, have lost the whole gene and their breakpoints require fine mapping at sites outside the gene before any further analysis can be attempted. To do this we have utilized anonymous sequences (derived from the human genome programme) mapped to the *HPRT* gene region, Xq26, to probe for presence/absence of defined sites in the mutants. In some cases we have sequenced these probes to enable PCR primers to be made for rapid testing of the presence of a site. Thus, we are building up a map of the location of the breakpoints for a set of mutants, and adding to this as new probes become available. In one complex X-ray mutant with a very large deletion (>1.5 Mb) and a translocation in Xq26 we have tentatively identified a breakpoint within one flanking site.

While continuing to work on the X-ray induced mutants, we have also isolated a series of mutants induced by α-particles from 238-plutonium. These have been classified, using a battery of PCR primers covering the *HPRT* gene, into those with large deletions and/or rearrangements and those with no observable change (presumed point mutations). The breakpoints of a number of the deletions have been mapped internally to the gene, and some have also been mapped with flanking probes (see above). The breakpoint of one internal deletion was isolated by PCR methods and sequenced: this mutation once again showed the involvement of short direct sequence repeats in the formation of the deletion.

Publications

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Annual Progress Report CEC project F13P-CT920007

Head of project 6: Dr. C.M.P. Backendorf

Title: Interference of radiation with skin homeostasis

II. Objectives for the reporting period

It has now been firmly established that exposure of living cells to radiation results in rapid changes in gene expression. However, the physiological significance of these changes is still poorly understood. Skin is one of the most radiosensitive tissues of the human body and constitutes a major dose-limiting organ in radiotherapy. The molecular basis for this high radiosensitivity is largely unknown. The maturation of epidermal cells (keratinocytes) is a strictly regulated process, where cellular proliferation, differentiation and cell death have to be tightly coordinated (homeostasis). By following the expression of a novel class of genes, which are tightly regulated during each of these processes (SPRR gene family = cornified envelope precursors), we were able to show that radiation (UV and ionizing radiation) can severely disturb skin homeostasis *in vivo* and *in vitro*. In the present project it is our aim to identify and study the regulatory factors (transcription factors) involved in the normal regulation and radiation induced deregulation of SPRR genes in cultured human keratinocytes.

III. Progress archieved including publications

From the results obtained sofar, it appears that SPRR2 is subjected to both positively and negatively acting regulatory factors:

A POU domain protein is involved in SPRR2 regulation during keratinocyte differentiation.

The SPRR2 promoter, fused to the CAT reporter gene, has been analysed by deletion mapping. These experiments have convincingly shown that an octamer site, situated at position -100 in the SPRR2 promoter, is essential for the induction of this gene during calcium induced keratinocyte differentiation. Octamer sites are recognized by a large family of developmental regulators, the POU domain proteins which are strongly conserved during evolution. These transcriptional regulators have been found to control the generation and maintenance of differentiated cell phenotypes in many tissues. Until now 13 different POU domain factors have been identified and classified in 6 different classes.
Bandshift analysis with the SPRR2 octamer site revealed that in keratinocytes a single protein-octamer complex can be observed. Proteolytic clipping experiments aswell as specific antibodies showed that the protein involved was the OCT-1 POU domain factor. The binding of OCT-1 to its target sequence is however not modulated during calcium induced differentiation of keratinocytes. Also the amount of OCT-1 protein in the cell, aswell as the phosphorylation state of the protein does not change

during keratinocyte differentiation. Similarly, in crosslinking experiments, no evidence was obtained that OCT-1 is associated with a cofactor in human keratinocytes. Only a slight potentiation of OCT-1 binding to the SPRR2 octamer site was monitored after X-ray irradiation of cultured keratinocytes. However, no change in OCT-1 binding was found after UV irradiation. As a consequence no clear evidence has been obtained sofar, which would suggest a direct involvement of OCT-1 in the regulation of SPRR2. As a matter of fact the OCT-1 factor is certainly not specific for keratinocytes but is expressed in many cell types. Hence, it might be possible that in keratinocytes another POU domain protein, which is not detected by the bandshift assay is involved in SPRR2 regulation.
In order to look for such a putative factor we have used POU domain specific primers and screened a keratinocyte-specific cDNA library by using the PCR technology. This screening has resulted in the identification of a POU domain gene, which is different from OCT-1 and seems to be most related to the OCT-2 class of POU domain factors. The isolation of a full-size cDNA clone is underway and should allow us to analyse whether this "keratinocyte specific" POU domain factor is actually directly involved in the regulation of the SPRR2 gene. Sofar it is not clear why this factor is not detected by the bandshift assay.

The AP-1 family of transcription factors inhibits the expression of the SPRR2 gene.

Whereas the binding of OCT-1 to the octamer target sequence does not change after induction of keratinocyte differentiation (see above), a clear decrease in AP-1 binding activity was found in the same nuclear extracts. The decrease in AP-1 binding activity is concommittant with an increase of SPRR2 expression. These results prompted us to analyse whether the AP-1 transcription factors were directly involved in the regulation (inhibition) of SPRR2 expression. AP-1 binding activity is composed of either hetero- or homodimers of two families of transcriptional regulators: the JUN family contains the genes *c-jun, junB* and *junD*, whereas the FOS family contains *c-fos, fosB, fra-1* and *fra-2*. The AP-1 family of transcription factors can also form heterodimers with the ATF/CREB family of transcription factors.
Northern analysis showed that in keratinocytes AP-1 activity correlates best with *c-jun* expression. Consequently we analysed whether *c-jun* can directly influence SPRR2 expression. That this is indeed the case was shown in experiments where the SPRR2 reporter construct was cotransfected with a *c-jun* expression plasmid. In these experiments the SPRR2 reporter plasmid became insensitive to calcium induction, suggesting that increased levels of *c-jun* in the cell prohibited the regulation of the SPRR2 gene during keratinocyte differentiation. When however the *c-jun* gene was replaced in the expression plasmid by the *neo* gene, SPRR2 induction was readily measured after increasing the extracellular concentration of calcium. From these experiments the following model is proposed: In proliferating basal cells the AP-1 binding activity is high and the SPRR2 gene is actively repressed. When these basal cells are induced to differentiate, AP-1 levels drop and the SPRR2 gene will be expressed. Preliminary experiments (in

collaboration with Dr. H. Bos (University in Utrecht) indicate that the calcium signal, responsible for AP-1 downregulation and SPRR2 induction, is channelled through the RAS proto-oncogene.
The model of SPRR2 regulation, proposed above, is further substantiated by UV irradiation experiments. Irradiation of basal (proliferating) keratinocytes results in an inhibition of SPRR2 induction and is concommittant with a strong increase in AP-1 binding activity. This response seems to be specific for UV irradiation. Indeed, if the same experiment is performed with X-ray irradiated basal cells, no variation in AP-1 binding activity is observed and SPRR2 is still readily induced after increasing the extracellular calcium concentration.
Another direct link between AP-1 binding activity and SPRR2 expression is found in squamous cell carcinoma cell lines. Here the constitutive AP-1 binding activity is significantly higher than in normal cells and SPRR2 expression is very low.
It was interesting to determine which sequences in the SPRR2 promoter were responsible for AP-1 mediated repression. First we analysed a sequence situated at position -200, which matches completly with the AP-1 binding site in the collagenase gene. However this sequence does not seem to be involved, because an SPRR2 construct where this sequence has been deleted is still repressed by *c-jun*. However, a construct were the region between -100 and -70 (just downstream of the octamer site at position -100) has been deleted, is no more repressed by *c-jun*. The -100/-70 region contains four direct repeats separated by either 8 or 9 nucleotides. Allthough this repeated sequence has some homology to an AP-1 binding site, it does however not conform to any known AP-1 site or to another known transcription factor binding site. As a consequence we do not know at present whether *c-jun* binds directly to this region, or whether the repetitive sequence is recognized by a novel transcription factor which is regulated by AP-1. Experiments are underway to clarify this point.

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Head of project 7: Dr. F. Eckardt-Schupp, GSF-Forschungszentrum Neuherberg

II. Objectives for the reporting period:

Our main objective is to measure the quantity and distribution of DNA double strand breaks (DSB) being either induced by ionizing radiation or enzymatically processed from S1 nuclease-sensitive sites (SSS) in the chromatin of mammalian and yeast cells. For this purpose we develop techniques based on pulsed field gel electrophoresis (PFGE) as well as computer-based evaluation procedures.

During the **period 1992/93** we have addressed the following topics:
- Development of computer analysis of DNA profiles in PFGE gels for the assessment of DSB in yeast.
- Search for experimental models for deviations from random distribution of DSB in yeast.
- Repair studies (rev2 mutant) applying the computer-based PFGE technique
- Development of a PFGE technique for mammalian cells suitable for computer-based evaluation.
- Cloning and molecular analysis of the REV2 gene of yeast.

During the **period 1993/94** we want to shift our research interests progressively. We plan to put more efforts in the analysis of DNA damage in mammalian cells and reduce the research on yeast systems. In detail we want to achieve the following goals:
- Development of computer analysis of profiles of radiation-induced DNA fragments of mammalian cells.
- Application of the method: analysis of various conditions of irradiation, repair and cell-cycle studies with mammalian cells.
- Analysis of induced and non-repaired SSS with special emphasis on chromosome-specific, non-random events in yeast.

III. Progress achieved including publications

1. Computer analysis of DNA profiles in PFGE gels of yeast

In the yeast S. cerevisiae all intact chromosomal molecules and their radiation-induced fragments can be separated according to size by PFGE. Ethidium bromide-staining of the gels and measurement of the fluorescence intensity distribution yields DNA mass profiles which comprise the peaks of the intact chromosomal molecules and fragments of heterogeneous length. Originally, DSB determination was performed by comparing the fluorescence intensity of distinct bands in treated and untreated samples allowing an estimation of the frequency of DSB induction.

However, for exact quantitation and analysis of distribution of induced and/or non-repairable damage in the yeast genome we improved our technique considerably. First, the distribution of DNA mass in the gel lanes versus migration distance is monitored as light intensity emitted during UV irradiation of the stained gels by use of a CCD camera and an image analysis device. Next, on the basis of a random breakage model by using

arbitrary values for the frequencies of DSB per unit length DNA mass distributions are calculated. These distributions are transformed into distributions in migration distances, based on a calibration of the positions of the chromosomal peaks in the gel and their known size. The shape of the peaks can be simulated assuming a Lorentzian distribution. Finally, the calculated profiles are normalized to the same area as observed profiles; the two profiles are compared using a least square procedure. The value for the DSB frequency yielding the best fit between observed and calculated distribution is determined.

For sparsely ionizing radiation a frequency of DSB per 10^9 Gy x bp was determined as 0.97 (± 0.08 SE) when irradiated under hypoxic conditions, and 2.57 (± 0.11 SE) under oxic conditions. To validate the new technique, pulsed field gels were blotted and hybridized to chromosome-specific gene probes. The frequency of DSB as calculated from the diminution of the hybridization signals in the chromosomal bands compare well to those obtained by the other method. However, both techniques yield smaller values than obtained by conventional techniques, i.e. centrifugation and elution.

2. Search for non-randomness of DNA damage distribution

The possible influence of DNA sequence and chromatin structure on the induction and repair of radiation-induced DNA damage is of increasing interest. Furthermore, a possible non-randomness of DNA damage distribution would have an impact on models for risk estimation.

From PFGE analysis of DSB induced in yeast DNA by rarely cutting restriction enzymes we have some preliminary evidence that our technique can detect deviations from DNA profiles based on random DSB distribution. So far, we have analysed the distribution of DSB induced under hypoxic as compared to oxic conditions of irradiation with sparsely ionizing radiation. We found no indication that under oxic conditions presumably radical-induced DSB differ in their distribution from those derived from direct energy disposition as proposed for hypoxic conditions of irradiation. In future, the distribution of DSB induced by neutrons and heavy ions will be analysed, which might show profiles of fragments indicating deviations from random distribution.

Our main long-ranging aim is the analysis of the quantity and distribution of SSS, a type of DNA damage which was considered to be distributed non-randomly. In accordance, several authors have reported that SSS are induced by ionizing radiation up to twice as frequently as DSB. Therefore we regard DSB as "model damage" which is much easier to handle experimentally and serves as internal standard for SSS measurements. At the moment the experimental procedures for the S1 nuclease digest are being optimized.

3. Repair studies

As we are particularly interested in a possible deviation of the distribution of remaining damage following repair conditions from randomness, it was important to test whether our computer analysis is suitable for the evaluation of gels obtained in repair studies, for instance liquid holding recovery (LHR). As test model we have chosen the diploid radiation-sensitive rev2 mutant of yeast and its repair-competent

wild-type. As opposed to mutants of the RAD52 epistasis group of repair genes, rev2 mutants are X/τ ray-sensitive though they are recombination-proficient, and they are capable of increasing their surviving fraction during LHR.

Our computer-based gel evaluation was suitable to prove that the X ray-sensitive rev2 mutant is capable to perform DSB repair during LHR, which is opposed to the phenotype of the "normal" X ray-sensitive yeast mutants. Now, besides having the evidence that our technique is suitable for repair studies, we want to study the interesting phenotype of the rev2 mutant. It is explicable either by a high percentage of error-prone DSB repair which could explain the increased sensitivity and the LHR capacity being reduced as compared to the wildtype strain. This however, cannot be visualized by PFGE. We have started to analyse the fidelity of DSB repair using a suitable plasmid system which will allow sequence studies; work is still in progress. Alternatively, we have indications that the rev2 mutant is defective in the repair of SSS. Once the experimental conditions for the S1 nuclease digest are optimized, we will analyse the SSS repair in wild-tpye and rev2 mutant strains.

4. PFGE technique for mammalian cells

We intend to establish a PFGE technique for mammalian/human cells which finally will allow to determine the induction and repair of DSB and SSS in various regions of the genome differing in structure and function. In particular, we want to achieve a technique where computer analysis is applicable, which seems not to be the case for the methods published so far. Certainly, since mammalian chromosomes are far too big to run into the gel (only fragments do), the approach has to be different from the one established for yeast.

In our proposal we intended to define fragments obtained by rarely cutting restriction enzymes, separated by PFGE and identified by suitable gene probes. This approach is not suitable for computer-based quantitation, so far. With probes for repetitive sequences, the hybridization signals for defined bands are distinct, however their quantitation bears difficulties. The number of the repeats in the various bands are unknown and furthermore, the smear induced by radiation is superimposed by comigrating small fragments derived from the bulk DNA remaining in the wells, which hybridize to the probes as well. In theory, this problem could be solved by using probes for single copy genes; in practice, the hybidization signals are not sufficiently strong because of the short probes we use so far. We would need large probes or a "population of probes" for defined regions of the genome. For trying this approach we would need support by other groups, supplying these special probes.

At the moment we have a completely different approach which resembles somehow the yeast technique. We separate the breakage-derived fragments migrating into the gel and obtain defined DNA mass profiles. Using suitable markers for the calibration of the migration behaviour of the fragments heterogeneous in length and applying the random breakage model we have begun to simulate the DNA mass profiles. This approach needs to be optimized, but we hope that finally it can be used to calculate the DSB frequencies and analyse the distribution of DNA damages.

5. Cloning and molecular analysis of the REV2 gene of yeast.

We are particularly interested in studies on the regulation of this repair gene of S. cerevisiae. Detailed biological analysis of the dose-effect curves for survival and mutation induction let us postulate an UV-enhanced expression of the gene reflected by certain characteristics of the kinetics.

Therefore we cloned the REV2 gene by complementation of rev2 point mutants, and sequenced it. The genomically disrupted gene caused the expected phenotype. In parallel, the gene (named RAD5 according to a different nomenclature) was cloned and analysed by two cooperating groups in the states [Johnson, R. et al. (1992) Molec. Cell.Biol. 12(9), 3807-3818]. Unexpectedly, the two ORF's differ in certain restriction sites due to frequent base pair exchanges and in their length. These differences are investigated by comparing the clone with the corresponding genomic region in various strains.

As our main interest lies in the regulation of the gene we have chosen a suitable repair-competent wild-type strain to identify the transcript. Obviously the gene shows low expression, however, by isolating mRNA from large batches of total RNA we succeeded in clearly demonstrating a transcript of 3,8 kb approximately. To prove the enhanced expression of the gene in response to UV and other stressing agents, we use RNA-PCR. Preliminary results indicate that at a dose of 80 Jm^{-2} the transcript levels are significantly enhanced.

List of publications relevant for the project

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Progress Report

Contract : **F13-CT920011** Sector : B13

Title : **Cytogenetic and molecular mechanisms of radiation myeloid leukemogenesis in the mouse.**

1) Janowski VITO
2) Cox NRPB USAF
3) Huiskamp ECN

I. Summary of Project Global Objectives and Achievements

At NRPB the principal objectives of the work are to gain an understanding of the cytogenetic and molecular mechanisms that underly the induction of the chromosome (ch)2 deletions and rearrangements which are believed to initiate acute myeloid leukaemia (AML) in irradiated mice. Also, to resolve possible genetic factors influencing predisposition to radiation leukaemogenesis.

Good progress has been made in relating radiation-induced ch2 breakage/rearrangement to chromosomal sites rich in a specific form of telomere-like repeat (TLR) sequence and a molecualr model to account for preferential damage expression at such sites has been developed.
Predisposition to AML in CBA mice has been linked with specific variation in germ line TLR sequences. Although technical problems have been experienced in the molecular cloning of the TLR locus of interest, good progress has been made in a study of germ line TLR variation in other inbred mouse strains.

At VITO the first objective is to complement the work done at NRPB by screening radiation AML predisposed and non predisposed mouse strains for TLR polymorphism. A second objective consists in attempts to induce mouse embryonic stem cells into haemopoiesis, in order to use them as a link between in vitro cell differentiation and in vivo leukaemia progression for gaining insight into the molecular basis of individual radiosensitivity. Finally, the unexpected features of radiation-induced non T leukaemias of the NFS mice could let us foresee a new mouse model for non AML leukaemias, representative of those that recently were revealed in A-bomb survivors.

Technical difficulties arose while trying to reach the first objective, but are presently overcome. Mouse embryonic stem cell cultures were established and first attempts were made in order to induce them into haemopoiesis. The radiation non T NFS leukaemias were characterised as biphenotypic myeloid/pre-B and may be considered as a new mouse model for the correponding human leukaemias.

At ECN the principal objectives of the work will focus on the induction of AML and other types of tumors in male CBA/H or hybrid mice after exposure to X-rays or 1 MeV fission neutrons in relation to exposure rate. Induced AML's will be histopathologically characterized and their in vivo malignant characteristics determined using in vivo passage procedures. DNA samples from AML's and normal tissue will be pre-processed for TLR sequence polymorphism determination at NRPB. In addition, to investigate whether genetic predisposition plays an important role in radiation leukemogenesis by irradiating CBA/H mice that were selected on basis of their TLR sequence polymorphisms. To investigate the effect of dose protraction on radiation induced carcinogenesis, all animals will be kept during their lifespan and be analyzed

for survival and tumor incidence in relation to dose rate with high and low LET radiation.

During the reporting period, 450 of the projected 590 male CBA/H mice have been exposed to various irradiation conditions. During the course of the experiments AML's are developing and 4 AML's have been processed for DNA analysis.
Genetically divergent mice have been mated and the F1 progeny will be irradiated during the coming month.

Head of Project 1 : Dr. M. Janowski

II. Objectives for the reporting period :

1. Screening radiation AML predisposed and not predisposed mouse strains for TLR polymophism

2. Initiating studies on myeloid differentiation and mutagenesis in embryonic stem cells (ES)

3. Characterising radiation non-T leukaemias of the NFS mouse strain

III. Progress achieved including publications

1. Attempts were made to analyse TLR polymorphism in the following strains : AML-sensitive SLJ/J (38 animals) and AML-insensitive CBA/Mol (37 animals, Balb/c (25 animals) and C57BL (30 animals).

DNA from normal spleen of the different animals was extracted using the conventional procedures and stored at -20°C. Digestion of the DNA's with the restriction enzyme Sau3A were performed according to the manufacturer's (GIBCO-BRL) instructions, and subjected to electrophoresis through 0.8% agarose gels for 16hr. DNA fragments were transferred to Gene Screen Plus filters and hybridized at 48° C with a labeled $(TTAGGG)_4$ synthetic oligonucleotide probe.

The restriction endonuclease Sau3 was found to generate a complex ladder of fragments hybridizing with varying intensity to the telomeric probe. In agreement with the results of Cox et al. the majority of these fragments were either > 50 kb or < 2 kb. TLR polymorphism was never observed in the zone between 4 and 8 kb. In order to check for a partial degradation of the DNA due to storage at -20° C, we analysed 4 CBA/Mol DNA's that had not been frozen but kept at 4° C. In these samples we could clearly detect different patterns in the zone of interest, but none of the patterns corresponded to those found by Cox et al. Analysis of a greater number of non frozen samples is needed to determine the different possible TLR polymorphism.

2. By using the multipotential embryonic stem cells (ES) we want to study the cellular consequences of specific leukemogenic events. ES cells are unique in the sense that they can be maintained in vitro as totipotent cell lines but also be experimentally committed to a variety of differentiation pathways, including engagement into the blood cell lineages. They could therefore be used as a link between in vitro cell differentiation and in vivo leukaemia progression, in order to gain insight into the molecular basis of individual radiosensitivity.
Undifferentiated ES cells were maintained as such by growing them on feeder cells. In this condition, they expressed the SSEA-1 antigen. They were than maintained in an adequate culture medium without LIF factor thereby loosing their SSEA-1 expression. Under these conditions, they differentiated sponteaneously as chaotic cultures. In order to classify them they were firstly stained with anti-SSEA-1 to avoid interference with the feeder cells, which are negative for SSEA-1. Secondly they were stained with differentiation-specific monoclonals (cytokeratin, vimentin, myosin, neurofilament) or with lineage-specific markers (approximately 20 were used) and analysed using a fluorescent activating cell sorter (FACS). Significant changes were not detected within the first 10 days of chaotic culture induction.
After a month, the ES cells could be classified into three cell populations (SSEA-1 bright, dull or negative), and they became slightly positive for haemopoietic lineage-specific markers : Sca, Thy, MAc3, c-kit. In order to induce ES cells into haemopoiesis and since c-kit is expressed on haemopoietic progenitor cells, positive ES cells for c-kit were isolated by FACS and tested for the capacity to form CFU-GM's. No CFU-GM could be detected, suggesting lack of

haemopoietic activity in the c-kit positive ES cells.
By adding haemopoietic stimulating growth factors like IL-3, M-CSF in the chaotic culture we hope to induce those cells into haemopoiesis.

3. Fractionated X-irradiation (4x1.75 Gy at weekly intervals) yields T cell lymphomas in 2/3 of the NFS mice. Other mice develop non T leukaemias. Eight of these were thoroughly characterized in collaboration with the University of Liège (Dr. M.P. Defresne) and Brussels (Dr. P. Robberecht), using electron microscopy and a large assay of cytochemical, immunochemical and molecular markers for lineage and maturation stage :

- Sudan Black, Prussian Blue, PAS, myeloperoxidase, non-specific esterase, chloroacetate esterase, acid phosphatase, alkaline phosphatase.
- GR-1, Sca-1, Joro38, pgp1 (CD44), T200 (CD45), HSA, LFA-1, Thy-1, CD4, Ly-1 (CF5), CD8, TdT, sIg, cIg, 6C3, IL2R, FcRII.
- Expression of IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, IL-10, TNFα, TNFβ, INFγ

The non T leukaemias were shown to consist of very early pre-B cells displaying simultaneously myeloid features, and may be considered as a new model for the rather common biphenotypic myeloid/pre-B leukaemias. They deserve special attention in view of the fact that a recent update of the leukaemia types among the A-bomb survivors demonstrates that the morphological picture for the A-bomb induced leukaemias is actually indistinguishable from that of the de novo human leukaemias, the proportion of the various leukaemia types being a function of the radiation dose and of the time of their appearance.

Publications

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Head of project 2 : Dr. Cox

II. Objectives for the reporting period

During the reporting period the objectives at NRPB were to seek further information on the possible involvement of interstitial telomere-like repeat (TLR) sequence arrays in the expression of ch2 damage, to continue work on the production of a ch2 "painting" libratry, to investigate the role of a polymorphic germ line TLR locus in predisposition of CBA mice to AML and to extend these TLR sequence analyses to other inbred mouse strains.

In the next reporting period molecular cytogenetic studies will be continued with an emphasis on the cloning of ch2 sequences, the development of fluorescence in situ hybridization (FISH) procedures for ch2 painting, the characterisation and mapping of ch2-encoded TLR sequence arrays and the investigation of possible inter-animal variation in the chromosomal radiosensitivity of CBA mice. A collaborative study of the chromosomal radiosensitivity of p53-deficient mice will also be initiated. Molecular studies on genetic predisposition to AML will focus on the cloning, mapping and inheritance of the TLR locus of interest and on TLR variation and AML predisposition in C3H mice. Work will also be initiated on $(CA)_n$ repeat sequence polymorphism for critical regions of ch2 to investigate DNA losses in AMLs expected from irradiated CBA x C57Bl hybrid mice (ECN Petten). Studies with Petten will also include CBA AMLs induced by low dose rate radiations.

III Progress achieved including publications

Cytogenetic and FISH studies

Cytogenetic and FISH studies have strengthened the link between AML-associated ch2 breakage and TLR sequence arrays having a non linear, inverted telomeric structure, ie. $(TTAGGG)_n$ ----- $(CCCTAA)_n$. The specificity of such sequence arrays to interstitial chromosome regions was further evidenced by the finding that a large (± 10 kb) synthetic linear telomeric probe, ie. $(TTAGGG)_n$ whilst highly efficient at labelling terminal regions showed poor labelling at interstitial sites. On the basis of these data a molecular model centred on stem-loop formation and guanine quartet bonding of inverted TLR arrays has been developed in order to explain the radiosensitivity of these potentially recombinogenic chromosomal sites. Work was also initiated on the FISH mapping of these arrays in human lymphocytes and on the possible association between terminal telomeric lenght and chromosomal radiosensitivy.

Molecular studies had suggested the possibility that TLR variation in CBA mice might be associated with differences in chromosomal radiosensitivity as measured by the frequency of stable aberrations during post-irradiation haemopoietic repopulation. A large study to investigate this has been initiated and although many of the mice have been cytogenetically scored the results, including DNA analyses, will not be available until next year.

Cytogenetic studies would be greatly facilitated by FISH painting of ch2 and to this end work has commenced using a recently acquired microdissection system and improved microcloning procedures.

Molecular studies on AML predisposition

The association between AML predisposition and locus-specific TLR polymorphism has been extended by the analysis of additional AML-bearing mice and a pilot study on the irregular inheritance of these polymorphism was completed. Much effort has been expended on the molecular cloning of the TLR locus of interest but, not unexpectedly, the inherent

instability of TLR-rich sequences in cloning vectors and the resistance of the target DNA to PCR amplification has slowed progress. Good progress has however been made in the investigation of TLR variation in other mouse strains using the Sau 3A digestion system developed for CBA. Results to date indicate that AML-insensitive C57BL mice do not show obvious TLR variation while variation is evident between mice from the AML-sensitive C3H colony in Chiba (Japan). The patterns of variation seen in CH3 for DNA fragments of <10 kb may be explained by cycles of expansion and contraction of TLR arrays; this possibility is being further investigated. Ongoing collaborative studies with Chiba are aimed at establishing whether a specific form of C3H TLR variation is associated with AML predisposition and whether C3H AMLs show characteristics TLR sequence changes.

Pulsed field gel electrophoresis (PFGE) techniques have been used to provide preliminary evidence of TLR variation in much larger (>100 kb) SalI restriction fragments of CBA mice. Current PFGE studies are centred on the ch2 TLR locus believed to be closely linked with the interleukin-1 gene cluster in order to determine whether the genomic changes to AMLs that are known to occur in this region might be associated with the germ line TLR polymorphism noted above.

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Head of Project 3 : Dr. R. Huiskamp

II. Objectives for the reporting period

During the reporting period, the objectives at ECN were to irradiate male CBA/H mice with different radiation qualities at various exposure rates to investigate the influence of these parameters on radiation induced carcinogenesis. Studies are focussed on induction of acute myeloid leukemia (AML) and other tumors during the animals lifespan. In addition, animals of the CBA/H colony will be screened for their telomere-like repeat (TLR) sequences and selected phenotypes will mated and irradiated to investigate the possible involvement of genetic factors influencing predisposition to radiation leukemogenesis. Genetically divergent mice will be mated and their F1 progeny will be used to investigate AML susceptibility by means of analysis of critical ch2 sequence losses using $(CA)_n$ repeat sequence polymorphism performed by NRPB.

In the next reporting period, studies on in vivo carcinogenesis will continue with emphasis on the induction of AML's in CBA/H x C57Bl hybrid mice and in CBA/H TLRp homozygotes. In addition, transfer of molecular technology from NRPB to ECN will be established to perform molecular studies at ECN.

III. Progress achieved including publications

During the reporting period, 450 of the projected 590 male CBA/H mice have been exposed to the various irradiation conditions :

90 mice irradiated with 2 Gy X-rays at 600 mGy/min (projected number of mice, n=90)
140 mice were exposed to 2 Gy X-rays at 3 mGy/min (projected number of mice, n=220)
140 mice were exposed to 0,4 Gy 1 MeV fission neutrons at 100 mGy/min (projected number of mice, n=140)
80 mice were exposed to 0.4 Gy 1 MeV fission neutrons at 0.5 mGy/min (projected number of mice, n=140)

No animals were lost during the long exposure periods. 120 control animals have been entered into the study. Total number of controls will be 200. Irradiation will be continued until the projected numbers in the various experimental groups have been reached. During the course of the experiments AML's are developing and so far 4 AML's have been processed for DNA analysis. Animals will be followed during their lifespan. Results on AML yields, other tumor incidences and survival analysis are not expected to be available during the present contract period due the projected mean lifespan of the animals of at least 2 years.
Genetically divergent C57Bl and CBA/H mice have been mated and the F1 progeny will be irradiated with 3 Gy X-rays during the coming months. Total number of mice that will be entered into the study will be 400 irradiated mice and 200 control animals.
Researchers of ECN have visited NRPB and transferred techniques to ECN for DNA analysis. This collaboration with NRPB will continue in order to carry out part of the molecular characterization of AML's at ECN.

Progress Report

Contract: FI3P-CT920017 Sector: B13

Title: Studies on radiation induced chromosomal aberrations.

1)	Natarajan	Univ. Leiden
3)	Ortins	LNETI
4)	Bryant	Univ. St. Andrews
5)	Pantelias	NCSR "Demokritos"

I. Summary of Project Global Objectives and Achievements

This research proposal aims at clarifying some of the basic mechanisms involved in radiation induced chromosomal aberrations in mammalian cells. The experiments have been be designed to come up with an answer(s) regarding (a) the influence of biological factors, such as repair proficiency of cells and chromatin configuration on the radiation induced chromosomal aberrations, (b) the influence of radiation quality, i.e low LET radiation (X-rays, gamma rays), high LET radiation (neutrons, heavy ions) and auger electrons on the frequency of chromosomal aberrations, (c) the response at low doses and dose rates and (d) the influence of some of the above factors in the production of stable (translocations) in comparison to unstable (dicentrics, rings) chromosome exchange aberrations as detected by chromosome painting technique.

Some of the achievements during the reporting period are:

X-rays delivered at low dose rates decrease the frequencies of translocations (detected by FISH technique) and dicentrics to a similar extent, though the frequencies of translocations are always higher than that of dicentrics in both cases. Inhibitors of DNA repair (3 AB, araC) increase the frequencies of X-ray induced dicentrics, but not the translocations, indicating that there may be differences in the repair process leading to these two types of aberrations. Chromatin structure (hetero and euchromatin) appears to have a profound influence on the induction and repair of X-ray induced chromosome damage. In addition, radiation induced aberrations are not randomly distributed among the human chromosomes.

Attempts have been made to clarify the basis of radiosensitive mutants of Chinese hamster cell lines. In addition to the defect in repair of DNA DSBs,the radiation sensitive xrs 5 CHO mutant cells appear to be in the number ofless number of binding sites per ug of their protein extracts in comparison to that of the wild type CHO cells. In radiosensitive irs-1 cells, the initial frequency of radiation induced chromosome damage as measured by PCC was very similar to wild type V79 cells. The kinetics of rejoining of interphase chromosome breaks was also similar indicating that the increased radiosensitivity of irs-1 cells may be due to more mis-repair of DNA lesions in comparison to V79 cells.

X-irradiation induced chromosome fragments in human lymphocytes and chromatid breaks in CHO cells indicated that there is no capping of breaks by insertion of telomeric repeats at break sites.

Head of project 1: Prof. Natarajan

II. Objectives for the reporting period

Studies during the reporting period were aimed at:
1. Quantification of micronuclei, chromosome translocations (by FISH technique) and dicentrics in human lymphocytes following X-irradiation at different dose rates
2. Evaluation of the influence of inhibitors of DNA repair on the yield of translocations and dicentrics (by (by FISH technique) induced by X-rays
3. Evaluation of the influence of beta carotene on the yield of X-ray induced chromosome aberrations and micronuclei in human lymphocytes.
4. Evaluation of the influence of chromatin structure on the induction and repair of X-ray induced chromosome lesions in Chinese hamster ovary cells.
5. Distribution of telomeres in X-ray induced chromosome fragments in human lymphocytes.

III. Progress achieved including publications

1) X-ray induced chromosomal aberrations in mammalian cells:

a) Influence of dose rates:

In our earlier studies we have shown that in human peripheral blood lymphocytes the frequencies of chromosomal translocations induced by X-rays are about 1.5 to 2 times higher than that of dicentrics. This difference was not found when lymphocytes were irradiated with 1 MeV neutrons (Natarajan et al, 1992 a,b). In order to probe further into the mechanisms of formation of radiation induced translocations, we have studied the influence of dose rate on the yield of micronuclei in binucleated i.e., cytokinesis blocked lymphocytes, dicentrics and translocations following X-irradiation and neutron irradiation. For induction of micronuclei 5 dose rates, ranging from 0.2 to 7.2 Gy/min of X-rays (3 Gy) were used. There was an initial reduction in the frequencies of micronuclei by 31 % at 2 Gy/min. and further reduction up 35.5 % and this reduction did not vary very much between different lower dose rates. For 1 meV neutrons, two dose rates, namely 0.05 Gy/min and 0.1 Gy/min and a dose of 0.5 Gy were employed. Blood samples from three probands were used for this experiment and there were no significant differences between the two dose rates in the yield of micronuclei. An RBE value of about 4 was calculated for induction of micronuclei by neutrons. For the evaluation of the yield of dicentrics blood samples from three donors were used. 4 dose rates (0.2 to 7.0 Gy/min) and a total dose of 3 Gy of X-rays were employed. One donor did not exhibit any dose rate effect. In the other individual there was no difference in the yield of dicentrics following two high dose rates (7 Gy and 2 Gy/min) but the frequencies were reduced by about 36% at the two lower dose rates (0.375 and 0.2 Gy/min.). The third donor showed a maximum reduction of 20% in the yield of dicentrics at the lowest dose rate (0.2 Gy/min). These data indicate that there could exist inter-individual variability with regards to dose rate effects for induction of dicentrics by low LET radiation.

Blood samples from two donors were used to assess the influence of dose rate on the yield of translocations for comparison. Two dose rates, namely 9.1 and 0.1 Gy/min and a total dose of 3 Gy of X-rays were employed. Conventional Giemsa stained preparations were scored for dicentrics and rings. For detection of translocations, fluorescent in situ hybridization (FISH) technique using DNA libraries specific for chromosomes 1,2,4 and 8 were used. The reduction in the yield of dicentrics and translocations was 43% and 23.3% respectively in one donor. In the other donor the values were 23.3% and 20%. In all cases the yield of translocation was 1.6 to 2 times higher than the yield of dicentrics.

b) Influence of inhibitors of DNA repair on the yield of X-ray induced dicentrics and translocations in human peripheral blood lymphocytes.

Earlier studies from our laboratory as well as others have shown that inhibitors of DNA synthesis or repair, such as aphidicolin, cytosine arabinoside (ara C), 3-aminobenzamide (3 AB) increase the yield of dicentrics induced in human lymphocytes by low LET radiation by a factor of about 2. It was of interest to check the influence of these inhibitors on the yield of X-ray induced chromosomal translocations. The inhibitors were added one hour before irradiation of the blood samples. 5 mM 3 AB or 50 μM ara C was used. The yield of translocations was higher than the yield of dicentrics following 1 and 2 Gy of X-irradiation. Treatment with ara C increased the frequencies of dicentrics by a factor of 1.8 to 2 in both the probands. 3 AB increased the frequencies of dicentrics by a factor of 1.4 to 1.7 in both the probands. However the frequencies of translocations detected by FISH technique were not increased by the inhibitors of DNA repair. These observations indicate that the translocations may be formed by a different mechanism from that involved in the formation of dicentrics.

c) Influence of radio-protectors on the yield of X-ray induced dicentrics and micronuclei

Beta carotene is known to protect chemically induced genetic damage in mammalian cells in vitro and in vivo. One of the mechanisms of action of beta carotene is scavenging free radicals. We investigated the ability of beta carotene to protect radiation damage in human lymphocytes. Lymphocytes were incubated for 2h in medium containing 0, 1, 2, 4 and 6 uM beta carotene and then irradiated with 0, 0.5, 1.5 or 3.0 Gy of X-rays and cultured for 48h and 72h (in the presence of cytochalasin B) to estimate the induced frequencies of chromosome aberrations and micronuclei respectively. B-carotene was found to reduce the frequencies of X-ray induced dicentrics and acentric fragments by 26 to 79% depending on the radiation and beta carotene doses. 2 uM beta carotene was found to give a significant protection. Similar reduction was also found in the frequencies of induced micronuclei. Similar experiments were carried out in Chinese hamster ovary cells (CHO) in which beta carotene was used as pre-treatment or post-treatment. The protection of radiation damage was evident only when the Beta carotene was given as pre-treatment indicating that the protection is mediated by interfering with events during irradiation and not due to post-irradiation such as DNA repair. In order to check whether the observed protection is due to scavenging of free radicals, human lymphocytes were irradiated with 3 Gy in the presence of air, oxygen or nitrogen in combination with beta carotene. The results indicated that maximum protection by beta carotene was obtained when the lymphocytes were irradiated in the presence of air or oxygen (63 and 31%) indicating that the protective action of beta carotene is due to prevention of indirect effects of radiation by scavenging of free radicals. The interesting observation is that beta carotene protects against radiation damage at very low concentrations (2-6 uM) in contrasts to other protectors like DMSO, which requires treatment with high concentrations (mM) to obtain an effect.

d) Influence of chromatin configuration on the yield of radiation induced chromosome aberrations

Primary Chinese hamster embryonic cells (CHE) contain a diploid chromosome number, namely 22 and all the 11 pairs of chromosomes can easily be identified. The pattern of distribution of heterochromatin among these chromosomes is very well characterized and the role of heterochromatin in the formation of chromosome aberration due to chemical treatment has been investigated by us (Natarajan and Schmid (1971) Chromosoma, 33, 48-52). In order to study the influence of heterochromatin on the induction and repair of radiation damage CHE cells were X-irradiated (1 Gy) and fixed at 1, 2, 3 and 4 h following exposure. Repair of DNA damage was completely blocked in one set of cells by 100 uM araA. The

frequencies of chromatid breaks and exchanges were evaluated for individual chromosomes. An analysis of observed and expected (on the basis of relative lengths of the chromosomes and assuming random induction of chromosomal damage) frequencies of chromosomal aberrations indicated that in general (a) the initial damage was higher in euchromatic regions than the heterochromatic regions and (b) the repair of DNA lesions (as evaluated by the frequencies of chromatid gaps and breaks) was more efficient in euchromatic regions than in heterochromatic regions.

e) Differential participation of human chromosomes in X-ray induced exchange aberrations

In order to clarify as to whether radiation induced chromosome aberrations occur randomly among the human chromosomes (expected on the basis of their DNA content) and whether individual chromosomes take part preferentially in translocations or dicentrics, lymphocytes (from 2 individuals) were X-irradiated with 1, 2, 3, and 4 Gy. The chromosome preparations were processed for conventional scoring of dicentrics as well as for fluorescent in situ hybridization (FISH) using chromosome specific DNA libraries. Six different chromosome libraries, namely # 1, 2, 4, X, 15 and 19 were employed. The comparison of different chromosomes indicated that DNA content is not the only factor influencing chromosome aberrations. Chromosome # 2 (7.9% of the genome), # 4 (6.2% of the genome) and # X (5% of the genome) participate to the same extent in aberrations. The frequencies of aberrations involving these chromosomes were higher than that observed for chromosome # 1 (8% of the genome). Chromosome # 15 (3.2% of the genome) participate in exchange aberrations to the same extent as chromosome # 1. These results point out that there is some non-randomness in the distribution of radiation induced chromosomal aberrations. In some chromosomes, such as # 1 and 2 the frequencies of translocations and dicentrics were very similar, whereas chromosomes # X and 15 were involved 2 to 3 times more in translocations than dicentrics. Some inter-individual variation for the participation of different chromosomes in aberrations were observed. In one individual chromosome # 4 participated in translocations three times more than in dicentrics, whereas in the other proband the frequencies involving chromosome # 4 in dicentrics and translocations were equal. Similar observation was also made for chromosome # 19. Further studies using blood samples from more probands and painting with more number of chromosomes are necessary to clarify this problem.

f) Distribution of telomeres in X-ray induced chromosome fragments.

Using telomeric probes we investigated the distribution of telomeres in human lymphocytes following X-irradiation. Three classes of fragments were observed, namely a) telomeric signals on both ends, b) telomeric signals at only one end and c) no telomeric signals. These types of fragments are expected. Fragments accompanying a dicentric usually have telomeres on both ends, whereas terminal deletions have one signal and interstitial fragments do not have any signals. There was no evidence for capping of breaks by telomerase, following X-irradiation.

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IV Objectives for the next reporting period;

1) Analysis of distribution of radiation (both high and low LET) induced translocations among different human chromosomes by FISH technique in order to check whether the translocations occur randomly or not. The results obtained will also allow us to decide as to which combination(s) of chromosome probes are most representative for the whole genome, an information necessary for biological dosimetry.

2) Mouse chromosome specific DNA libraries are being generated in our laboratory and these will be used to estimate the frequencies of dicentrics and translocations induced in vivo by X-rays and 1 meV neutrons.

3) The influence of intercalary teleomeric sequences on the formation of radiation induced chromosome aberrations in Chinese hamster, mouse and human cells will be studied.

Head of project 3: Dr. Ortins

II. Objectives for the reporting period

Studies of the adaptive response of human lymphocytes from low and highly exposed workers of Portuguese uranium mines.

1- Selection of uranium mines individuals according his historical report of work, as low or high exposed to radiation.

2- Establishing culture techniques for human lymphocytes and making metaphase slides to analyse the frequency of chromosome and chromatid aberrations, sister-chromatid exchanges and micronucleus, before and after, adaptive and challenge doses.

III. Progress achieved including publications

Uranium mines individuals were selected as high or low exposed based on the historical report of personal dosimetry.

Two groups of individuals not working on uranium mines with same age as miners were selected as match controls, one group living near mines homes and other living on a region with low level background of radiation.

Heparinized blood samples from each donor were collected by venepucture and whole blood cultures were made.

The peripheral lymphocytes blood cultures were set up in 15 ml glass bottles adding 0.5 ml of whole blood to 5 ml of RPMI 1640 medium containing 15% of new-born calf serum, 2 mM L-glutamine, 100 U/ml of penicillin, 100 μg/ml of streptomycin and 2% of PHA and kept at 37 ° C.

For each donor we look for adaptive response with 4 different end-points.

1 - Chromosome aberrations

Five hours after beginning of the cultures the lymphocytes in glass bottles were irradiated with a priming dose of 5 cGy from a Cobalt source gamma rays, and 5 hours later irradiated with a challenge dose of 300 cGy.

The lymphocytes were then cultured till 48 hours and for the last 2 hours the cells were treated with 1 μg/ml colcemid.

Lymphocytes were collected, treated with 75 mM potassium chloride for 10 min and fixed in 3:1 methanol acid acetic mixture.

Fixed cells were dropped onto clean slides, stained with Giemsa and coded slides analysed for chromosome damage.

2 - Sister chromatid exchange

The protocol for culture, priming and challenge dose was identical to the first one except that the cultures were set up in presence of 25 μM bromodeoxyuridine, cells harvested at 72 hours and fixed cells stained with the standard fluorescence-plus-Giemsa technique.

3 - Micronucleus

The protocol for culture, priming and challenge dose was identical to the first one except that the cells were harvested at 72 hours, and 24 hours before harvesting and fixation, Cytocatalasin B was added to a final concentration of 3.0 μg/ml. Fixed cells were stained with May-Grunwald-Giemsa.

4 - Chromatid aberrations

Five hours after beginning of the cultures the lymphocytes in glass bottles were irradiated with a priming dose of 5 cGy from a Cobalt source gamma rays, and 50 hours later irradiated with a challenge dose of 300 cGy.

The lymphocytes were then cultured till 72 hours and for the last 2 hours the cells were treated with 1 μg/ml colcemid.

Lymphocytes were collected, treated with 75 mM potassium chloride for 10 min and fixed in 3:1 methanol acid acetic mixture.

Fixed cells were dropped onto clean slides, stained with Giemsa and coded slides analysed for chromosome damage.

Till now, blood was collected from 10 miners and 8 controls and we did slides for analyse the frequency of chromosome and chromatid aberrations, sister-chromatid exchanges and micronucleus.

Publications:

Pereira Luís,J.H. And Póvoa V. (1992): Individual variability of adaptive response of human lymphocytes primed with low dose gamma rays. In: Low dose irradiation and biological defense mechanisms. Eds. T Sugahara, L.A. Sagan and T. Aoyama. Elsevier Science Publishers, pp 315-317.

Silva M.J.,Dias A.L.,Pereira Luís J.H., Carothers A and Boavida M.G. (1993): Dose-response relationship for gamma-radiation induced micronuclei in cytokinesis-blocked human lymphocytes. European Society of Human Genetics, 25th Annual Meeting, Barcelona, Espanha May 6-9.

Objectives for the next reporting period:

Following the standardization of the techniques to study chromosomal damage in Uranium workers and controls adaptive response experiments will be carried out using blood samples from these two groups of individuals.

Head of project 4: Dr. Bryant

II. Objectives for the reporting period

The main objectives of the project are the study of the mechanisms involved in conversion of specific types of DNA double-strand breaks (dsb) into chromosomal aberrations, especially in the radiosensitive G2 phase of the cell cycle. The work has focussed primarily on the chromosomal responses of radiosensitive mutants of Chinese hamster (*xrs5, irs2*) and radiosensitive human cell lines (AT) and the introduction of nuclear extracts into irradiated and restriction endonuclease treated porated cells. The molecular mechanisms of chromatid break formation was further investigated in hamster cells by examining the ends of visible breaks for the presence of new telomere sequencies (which would indicated covalent end-closure of breaks) using *in situ* hybridization with a telomeric probe.

III. Progress achieved including publications

Studies on nuclear extracts of xrs5 cells.
Protein extracts have been derived from CHO and *xrs5* cells. These proteins were extracted by a detergent free system involving the sequential washing of nuclei released by homogenisation with buffers of increasing ionic strength. Spermine hydrochloride (6mM) was also included to facilitate nuclear protein removal and maintain a compact DNA structure during extraction. The two ionic strength fractions examined so far are 100mM KCl and 250 mM $MgCl_2$. A protein binding assay involving the binding of ^{32}P labelled calf thymus DNA to the extracts was performed on extracts derived from non-irradiated cells and extracts from cells exposed to 10 Gy gamma rays followed by 30 minutes incubation at 37°C prior to extraction. The results from the non-irradiated fractions are shown below. The irradiated fractions gave similar results although both the 250 mM fractions were greater than the non-irradiated fraction. It is noticeable that the 100 mM extracts show non-specific linear binding to DNA whilst 250 mM extracts approximate to normal inverse exponential enzyme kinetics. Comparative regression analysis of the data gives the relative binding activity of *xrs5* fractions compared to CHO fractions (Table I). No difference is observed between the 100 mM extracts whilst the 250 mM extracts show a reduced DNA binding activity of *xrs5* proteins.

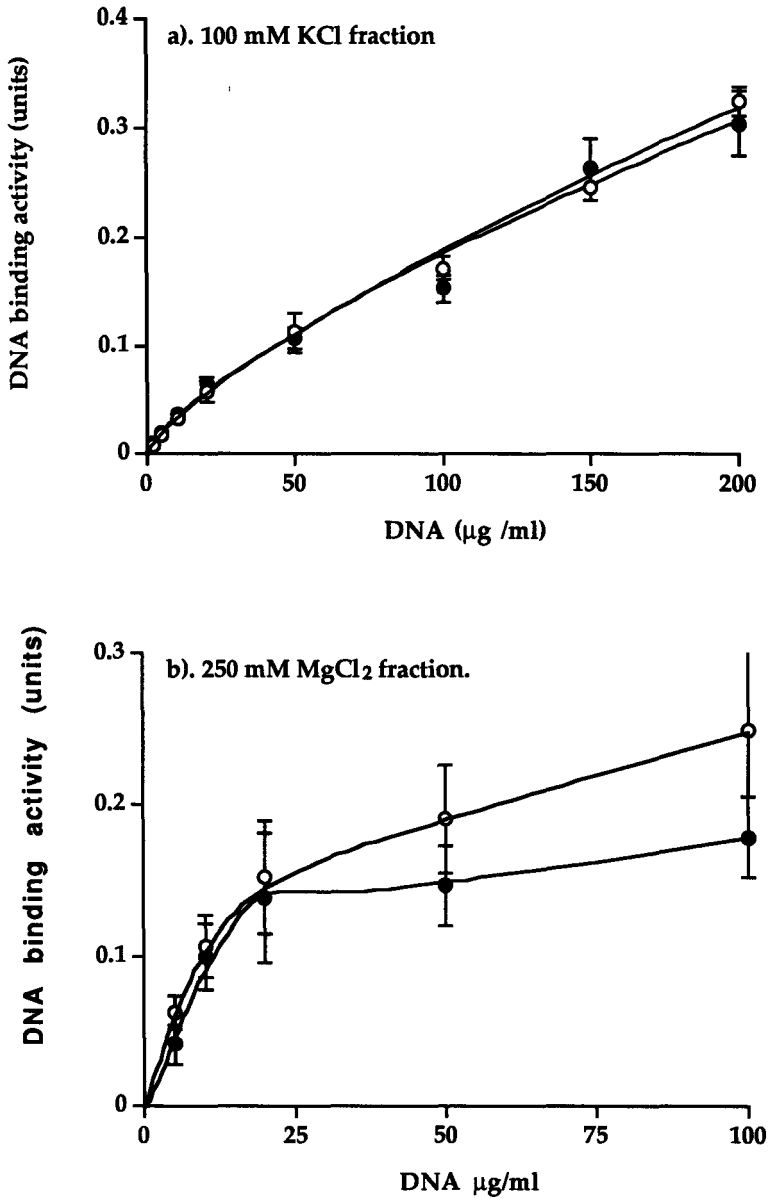

Figure 1. DNA binding activities of non-irradiated extracts of CHO (open symbols) and *xrs5* (closed symbols) versus an increase in the concentration off calf thymus DNA. a) 100 mM KCl fraction b) 250 mM $MgCl_2$ fraction. Error bars show standard error of the mean of 4-8 experiments.

	Relative DNA binding activity.	R^2	P
100 mM	0.938	95.3%	<0.001
100 mM (irradiated)	1.080	91.1%	<0.001
250 mM	0.724	71.4%	<0.001
250 mM (irradiated)	0.666	90.5%	<0.001

Table I. Relative DNA binding activity of *xrs5* extracts compared to CHO extracts. R^2 is the correlation coefficient and P is the confidence value of the analysis of regression. Figures derived from the correlation of individual data points from 3-7 independent experiments.

From Table II it is apparent that there are differences between CHO and *xrs5*. In addition to binding more DNA per μgof extract, CHO extracts have a reduced initial rate. This would indicate that the affinity of individual binding sites is reduced.

	▵ (units μg^{-1} ml^{-1})	V_{max} (units)	K_m (μg ml^{-1})
CHO	0.016 (±0.0029)	0.290 (±0.084)	18.58 (±3.66)
Xrs5	0.027 (±0.0115)	0.199 (±0.026)	14.95 (±5.93)
CHO (irradiated)	0.019 (±0.00200)	0.356 (±0.087)	18.57 (±3.38)
***Xrs5* (irradiated)**	0.037 (±0.0150)	0.241 (±0.050)	17.87 (±5.28)

Table II Kinetics of DNA binding by 250 mM extracts. Where ▵ = initial rate of binding, V_{max} is the maximum amount of DNA bound per μgextract, K_m = Michaelis constant which is the concentration of DNA required to give half V_{max}. Results show the standard error of the mean of 3-4 independent experiments.

Therefore it appears that CHO extracts have more DNA binding sites per μgthan *xrs5* extracts, but that these sites have a lower affinity for DNA. This results in the Michaelis constant showing only a slight variability between cell lines. These kinetic evaluations can only be taken as approximate since the extract fractions studied are still relatively crude and can not be expected to follow ideal Michaelis-Menten kinetics. This is apparent in the large errors associated with the values.

To asses the proteins responsible for the reduction in DNA binding activity a series of electrophoresis experiments were performed. Using separation by one dimensional polyacrylamide gel electrophoresis in the presence of SDS and 4M urea no obvious differences between cell lines could be observed for any of the extracts, except for relatively minor variations in quantity of some bands (figure 2a). The technique of South-western blotting was then applied to the extracts. This involves the blotting of extracts separated by electrophoresis onto nitrocellulose membrane. The membrane is subsequently incubated in a buffer containing a radio-labelled DNA probe. DNA binding proteins can then be visualised by auto-radiography (figure 2b). Extracts obtained by 100 mM KCl revealed limited low levels of DNA binding except at one prominant molecular weight (≈23 Kd). Little difference is observed between cell lines except in a faint band at ≈29 Kd where CHO would appear to bind more DNA than *xrs5*. Irradiation results in the increase in the binding at this molecular weight in both CHO and *xrs5*. 250 mM extracts produce several intense bands and other bands not resolved in this gel which remain in the stacking gel. Of the main resolved bands differences occur in several bands. There is a reduction in binding of DNA in the 29 Kd region obvious in *xrs5*. Interestingly on irradiation the levels of DNA bound increase in this band in *xrs5*. Irradiation also produces changes. In a band at ≈10Kd there is an increase in binding in CHO and a decrease in *xrs5* compared to the non-irradiated samples and an increase in both cell lines at ≈40Kd. In general *xrs5* shows a reduction in the apparent amount of DNA bound by certain bands which appear similar in quantity as measured by coomassie blue staining. Identification of the several proteins involved is still required. It is also planned to extend these studies to fractions obtained by increasing ionic strengths.

The possibility of several proteins being altered and the apparent reduction in binding affinity of these proteins would indicate the involvement of a defective control mechanism in *xrs5*. This control mechanism may be responsible for post-translational modifications which reduce DNA binding affinity. Since the proteins that are defective in *xrs5* would also appear to alter in binding affinity on irradiation there is a possible link between these proteins and the radiation sensitivity of *xrs5*.
To date attempts to complement the defect in *xrs5* by introduction of wild type proteins by streptolysin-O induced poration has been relatively unsuccessful. The identification of biochemically altered proteins may enable complementation to occur. Further work is planned in this area.

Studies on ataxia telangiectasia cells.
The chromosomal responses of lymphoblastoid AT cells to gamma-radiation and to the restriction endonucleases (RE) *PvuII* and *BamHI* have been studied in order to test the possibility that AT cells have a dsb processing defect. We have shown that RE treatment of porated human cells mimics the clastogenic effect of radiation in causing both break and exchange-type aberrations. Our results show that like gamma-rays, PvuII induces a higher frequency of aberrations in AT than normal cells (by a factor of 5) at both 5 and 24 h sampling times (figures 3a and b). Dsb induced by PvuII (blunt-ended) were found to be much more effective than cohesive-ended dsb in causing chromosomal aberrations, as found previously for rodent cells.

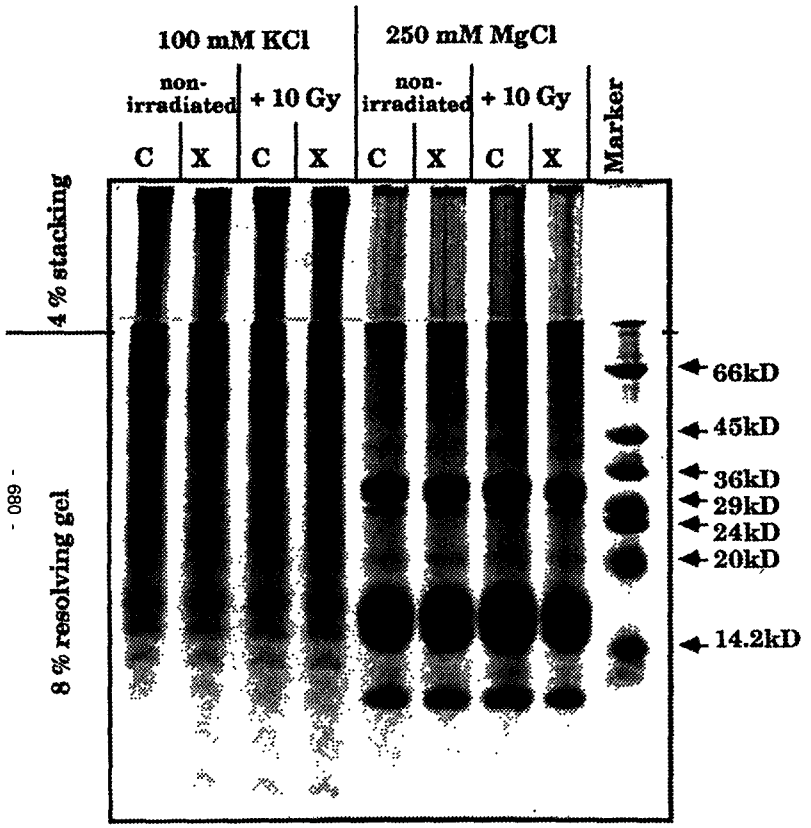

Figure 2 a) Coomassie blue stained electrophoretogram of extracts from CHO (C) and xrs-5 (X). Extracts shown are 100 mM KCl and 250 mM $MgCl_2$ fractions from non-irradiated and irradiated (+10 Gy) cells.

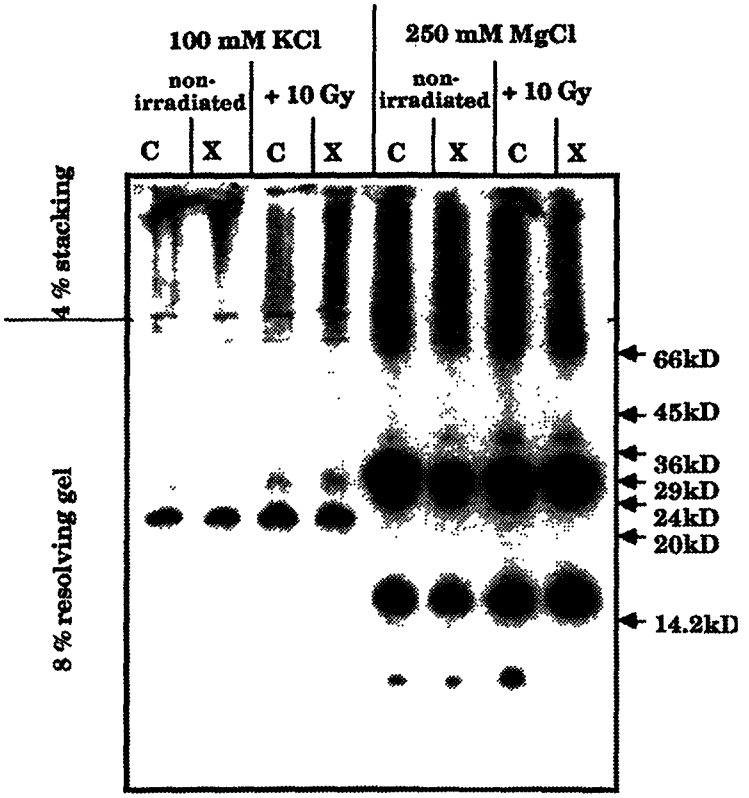

Figure 2 b) Autoradiogram of an identical gel to (a) which was blotted on to nitrocellulose and incubated with a random primed ^{32}P-labelled calf thymus DNA probe.

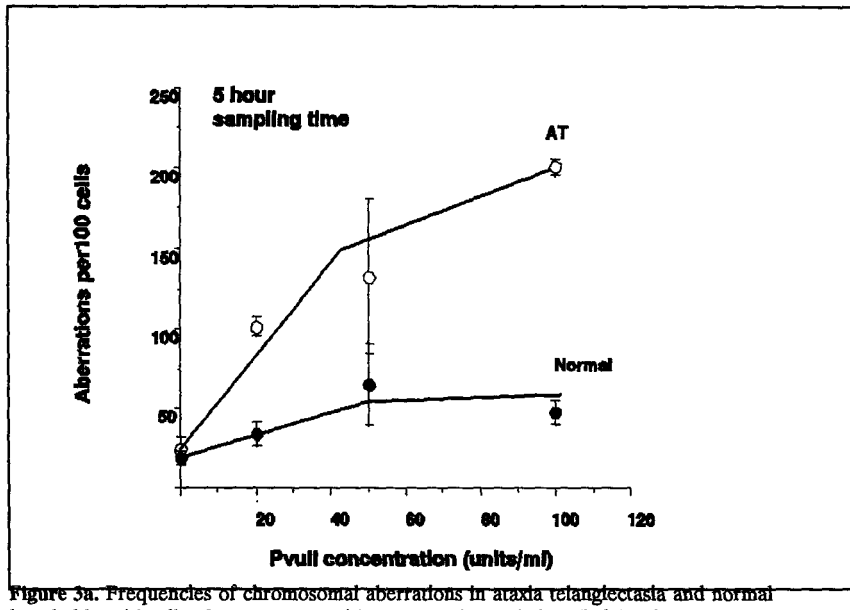

Figure 3a. Frequencies of chromosomal aberrations in ataxia telangiectasia and normal lymphoblastoid cells after treatment with PvuII, and sampled at 5h following treatment. (Redrawn from Liu and Bryant 1993).

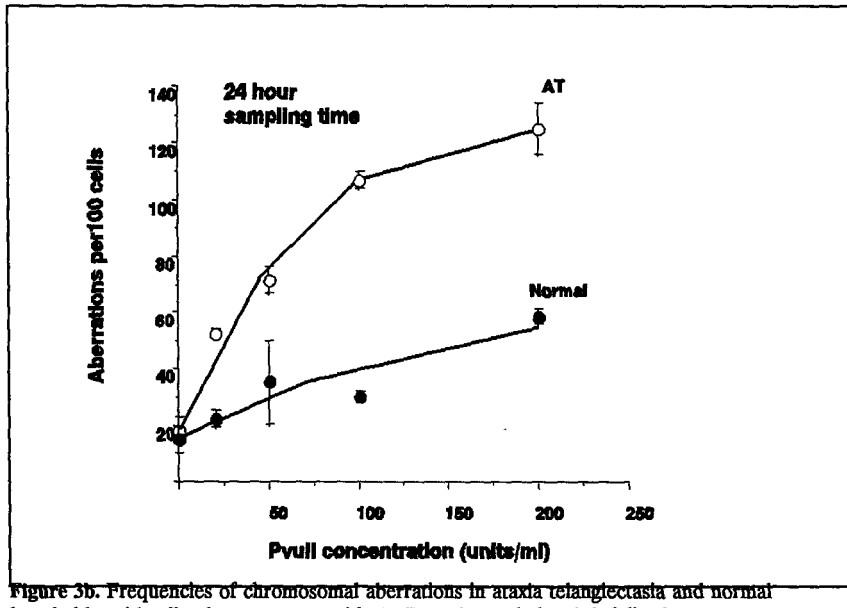

Figure 3b. Frequencies of chromosomal aberrations in ataxia telangiectasia and normal lymphoblastoid cells after treatment with PvuII, and sampled at 24h following treatment. (Redrawn from Liu and Bryant 1993).

Nuclear extracts were prepared form lymphoblastoid normal AT cells. These extracts have been tested for DNA ligase, nuclease, and topoisomerase activity using an in vitro plasmid assay. The extracts were shown to have strong topoisiomerase activity, but no detectable ligase or nuclease activity.

When nuclear extracts from normal cells were porated (using streptolysin O) into gamma-irradiated AT cells and the frequencies of chromosomal aberrations measured, no decrease was detected. However, when nuclear extracts were porated into cells treated simultaneously with Pvu II, frequencies of chromatid aberrations (principally breaks) were substantially lower than in cell porated and treated with PvuII alone (about a 2 fold decrease). When AT cells were treated with PvuII and nuclear extract from AT cells, no decrease in breaks was observed.

At this stage the nature of the protein or factor responsible for this decrease in aberrations is not known, however experiments are in progress to attempt to identify and characterize the proteins involved. Since the extracts showed no ligase activity in the in vitro assays we assume that the protein (or factor) in the extract is not a DNA ligase, however we are currently attempting reconstruction experiments involving both T4, calf thymus and human ligases.

Examination of chromatid breaks for evidence of telomeric end-closure.
It is possible that the mechanism of conversion of dsb into visible chromatid breaks involves covalent closure of the dsb termini, thus rendering them unrejoinable. Covalent closure might occur by the insertion of *de novo* telomeric repeat sequencies (TRS). We tested this hypothesis by examining the ends of chromatid breaks and gaps in metaphase chromosomes for the presence of TRS by using fluorescent *in situ* hybridization (FISH) with a biotinylated telomeric probe. Breaks in metaphase chromatids of irradiated G2 Chinese hamster embryo (CHE) and Chinese hamster ovary (CHO) cells were examined in this way. 100 metaphases containing chromosomes showing chromatid breaks and gaps were examined in each cell line. No evidence of chromatid break ends with associated telomeric sequencies was found. We conclude that if end-closure occurs at the termini of dsb in irradiated cells, either this process does not involve insertion of TRS's, or that the length of the TRS at break sites are too short to be detected using the FISH technique.

Publications over reporting period:

Bryant, P.E. (1992) Induction of chromosomal damage by restriction endonuclease in CHO cells porated with streptolysin. Mutation Research, 268, 27-34.
Macleod, R.A.F., and Bryant, P.E. (1992) Effects of adenine arabinoside and coformycin on the kinetics of G2 chromatid aberrations in X-irradiated human lymphocytes. Mutagenesis, 7, 285-290.
Bryant, P.E. (1992) Mimicking radiation with restriction endonucleases. British Journal of Radiology,Supplement 24, 35-38.
Bryant, P.E. and Johnston, P.J. (1993) Restriction endonuclease induced DNA double-strand breaks and chromosomal aberrations in mammalian cells. Mutation Research, 299, 289-296.
Johnston, P.J., and Bryant, P.E. (1993) Chromosome damage induced by nanomolar concentrations of bleomycin in porated mammalian cells. Biochemical Pharmacology, 45, 569-572.
Bryant, P.E., Jones, N.J. and Liu, N. (1993) Radiosensitive Chinese hamster irs 2 cells show enhanced chromosomal sensitivity to ionising radiation and restriction endonuclease induced blunt-ended double-strand breaks. Mutagenesis, 8, 141-147.
Bryant, P.E. (1993) Responses of radiosensitive mutant mammalian cells to restriction endonuclease induced dsb. In "Chromosome aberrations", G. Obe and A.T. Natarajan, Springer Verlag, in press.
Bryant, P.E. and Slijepcevic, P. (1993) G2 chromatid aberrations: kinetics and possible mechanisms. Environmental and Molecular Mutagenesis, in press.
Liu, N. and Bryant, P.E. (1993) Response of ataxia telangiectasia cells to restriction endonuclease induced DNA double-strand breaks: I. Cytogenetic characterization. Mutagenesis, in press.

Objectives for the next reporting period:

Studies on DNA binding proteins in xrs and wild type cells will continue, measuring the levels of protein phosphorylation as a function of passage through the cell cycle. DSB repair will also be studied in detail using filter elution technique. Influence of extracts of wild type cells on the chromosomal response of of restriction endonuclease treated AT cells will be studied.

Head of project 5: Dr. Pantelias

II. Objectives for the reporting period

1. To investigate whether there are differences between primary human normal and tumor cells in the induction of interphase chromosomal damage as visualised by means of the PCC method in G_0 or G_2 phase after X-irradiation.

2. To study whether the large difference in radiosensitivity between the mutant irs-1 and the parental V79 cell line is due to differences in the induction or repair of chromosome damage as visualised by means of the PCC method in plateau-phase cells after X-irradiation.

3. To study the mechanisms of hyperthermia-induced radiosensitization.

4. To develop biological dosimetry methods based on the analysis of peripheral blood lymphocyte prematurely condensed chromosomes (PCCs).

III. Progress achieved including publications

1. Radiation-induced chromosomal damage in primary tumor cells and in peripheral blood lymphocytes of cancer patients in comparison with that induced in normal cells.

It has been reported that human tumor cells or cells from cancer-prone individuals, compared with those from normal individuals, show a significant higher yield of chromatid breaks and gaps at metaphase after G_2 X-irradiation. Such an enhanced G_2 chromatid radiosensitivity could result from a) a greater radiation-induced initial damage; b) a greater inherent chromatid instability; c) a decreased radiation-induced G_2 block, allowing less time for DNA repair; and d) an impaired capacity to repair the DNA damage.

In an attempt to elucidate the mechanisms underlying the enhanced G_2 chromatid radiosensitivity, the PCC technique has been used by our group to study induction and repair processes of radiation-induced chromosomal damage directly in the G_0 and G_2 without having cells proceed to mitosis. In this way, the yields obtained are not subjected to cell cycle kinetics or other confounding factors of the conventional metaphase chromosome analysis.

Tumor specimens from human colon carcinomas were minced, digested for 1h with pronase, collagenase and deoxyribonuclease, and the cells were disaggregated for 0.5h with trypsin. In order to study the initial radiation-induced chromosomal damage in pure tumor cells, the cell mixture was then treated with CEA antibody binding, and sorted. The isolated cells were then irradiated in ice at various doses up to 4 Gy and fused immediately after with HeLa mitotic cells. A linear dose-response

curve was obtained and the initial yield of excess chromosome fragments per cell per Gy, in G_0 cells, was similar to that obtained in normal primary cells.

In other experiments peripheral blood lymphocytes from breast cancer patients were used to study the induction of initial chromosomal damage in G_0 as well as in G_2 phase. The yields obtained for chromosome and chromatid damage were found to be similar to those obtained in lymphocytes from healthy individuals.

Further experiments have been designed to investigate whether repair processes of radiation-induced chromosomal damage in normal and tumor cells are different. Short time periods up to 1h after X-irradiation will be allowed for repair before cell fusion and PCC induction take place.

2. Differences in induction and repair of radiation-induced interphase chromosomal damage between the radiosensitive mutant irs-1 and its parental cell line.

Induction and rejoining of interphase chromosome breaks were measured, after exposure to X-rays, in plateau-phase Chinese hamster V79 cells and in a radiosensitive mutant cell line derived from them, irs-1, using premature chromosome condensation (PCC). There was no difference in the induction of interphase chromosome breaks per Gy between the radiosensitive mutant cells and wild-type V79 cells despite the large differences in their radiosensitivity; an induction of 2.85 $\pm$0.05 breaks/cell/Gy was measured in both cell lines. Also, rejoining of interphase chromosome breaks proceeded in the two cell lines with similar kinetics ($t_{1/2}$ = 26.2 min). In contrast, ring chromosome formation was higher in irs-1 cells, as compared to wild-type V79 cells (0.78 versus 0.44 after 6h of repair). These results confirm previous observations suggesting that a general deficiency in the rejoining of DNA dsb is unlikely to be a direct cause of the increased radiosensitivity of irs-1 cells, and are consistent with the hypothesis that the increased radiosensitivity of these cells derives from an increase in the probability of misrepair.

3. Hyperthermia-induced radiosensitization

A large number of alterations have been observed in cellular structures and functions after exposure to heat and have been considered in the mechanism of heat-induced radiosensitization and cell killing. However, a direct causal relationship between alteration in nuclear architecture and the various effects of heat treatment was not possible since interphase chromatin and the nucleoli could not be visualised by means of conventional cytogenetic techniques.

Using the PCC method we have shown that exposure of interphase cells to elevated temperatures (42-45 ^{o}C) induces dramatic changes in chromatin conformation and a reduced ability of chromatin to condense and of the nucleoli to disintegrate under the influence of factors provided by the mitotic PCC-inducer cells.

The effects of such alterations in nuclear architecture on the induction and repair of radiation-induced interphase chromosomal aberrations have been thoroughly investigated (Pantelias and Iliakis, manuscripts in preparation). Plateau phase CHO cells pre-exposed to heat (45.5 ^{o}C) for up to 15 min in fresh growth medium without serum, were irradiated and either analysed immediately or returned to the incubator at 37 ^{o}C in their conditioned medium. At various times thereafter (up to 24 h), flasks were trypsinized and assayed by means of the PCC method for excess chromosome fragments. Alternatively, cells were first irradiated and subsequently allowed for repair either at 37 or at 41 or 42 ^{o}C for up to 6h. The results obtained clearly indicated a significant reduction in the ability of cells to repair radiation-induced chromosome damage if kept at elevated temperatures after irradiation. The experiments also indicated an increase in the induction by radiation of chromosome damage in cells heated before irradiation, but not significant alterations in the rate of repair. An increase in misrepair and ring chromosome formation, however, was observed as chromatin condensation was increased by increasing heat treatment time before irradiation. These results contrast with observations at the DNA level which show a dramatic reduction in DNA repair in pre-heated (45.5 ^{o}C) cells and no delay in DNA repair of incubated cells at either 41 or 42 ^{o}C after irradiation.

The modification by heat in the induction and repair of interphase chromosome damage parallels results obtained at the cell level under similar treatment conditions and indicate the possible importance of heat-induced chromatin alterations in the mechanism of heat-induced radiosensitization.

4. Use of PCC for biological dosimetry

The application of the PCC methodology for biological dosimetry purposes, as an alternative to the conventional metaphase technique, has been examined by our group for some time now. With the goal to develop a quick and sensitive approach, C-banded lymphocyte PCCs and, more recently, painted lymphocyte PCCs have been considered.

Experiments complementary to those carried out in the previous funding period (BI7-0039-C) confirmed again the use of C-banded peripheral blood lymphocyte PCCs for biological dosimetry purposes. Centric rings, dicentric chromosomes, and acentric fragments can be scored directly in G_0 lymphocyte PCCs within 3 to 4 hours after blood sample withdrawal.

It has been recently examined as well the possibility to use the PCC technique in combination with the fluorescence in situ hybridisation (FISH) methodology to analyse stable translocations in painted peripheral blood lymphocyte PCCs. For this purpose were used Whole Chromosome Painting probes (without biotin and streptavidin, GIBCO BRL) that contain unique sequences homologous to DNA sequences along the entire length of the target chromosomes. These probes are labelled directly with detectable spectrum orange fluorophores, eliminating thus the need for the time consuming indirect detection methods required by other systems. In fact, painted lymphocyte PCCs ready for analysis were obtained in our laboratory within only 24 hours form the moment a blood sample was drawn. Experiments are presently under way to score stable translocations in lymphocyte PCCs and to test whether this technique may be established as a reliable biological dosimetry approach.

Publications

Pantelias, G.E., G.E. Iliakis, C.D.Sambani, and G.Politis (1993). Biological dosimetry of absorbed radiation by C-banding of interphase chromosomes in peripheral blood lymphocytes. *Int. J. Radiat. Biol*, 63, 349-354.

Okayasu, R., G.E. Pantelias and G. Iliakis (1993). Increased frequency of formation of interphase ring-chromosomes in radiosensitive *irs-1* cells exposed to X-rays. *Mutation Research*, in press.

Pantelias, G.E. (1993). Factors determining the yields of radiation-induced chromosome aberrations as visualised by means of premature chromosome condensation in interphase cells. In Chromosomal aberrations: Origin and Significance, G. Obe and N.T. Natarajan (eds), Springer Verlag.

IV. Objectives for the next reporting period

1) To investigate, by means of the PCC technique, the mechanisms underlying enhanced G_2 chromatid radiosensitivity of human tumor cells and cells from cancer-prone individuals. It will be examined, in particular, whether repair processes of radiation-induced chromosomal damage in interphase normal and tumor cells are different.

2) To test whether scoring of stable translocations in lymphocyte PCCs, painted with whole chromosome probes labelled directly with detectable spectrum orange fluorophores, may be used as a quick, sensitive and reliable biological dosimetry approach.

Progress Report

Contract: **FI3P-CT920028** **Sector: B13**

Title: Radiation-induced processes in mammalian cells: principles of response modification and involvement in carcinogenesis.

1)	Van der Eb	Univ. Leiden
2)	Sarasin	CNRS
3)	Devoret	CNRS
4)	Rommelaere	DKFZ
5)	Bertazzoni	CNR
6)	Thomou-Politi	NCSR "Demokritos"
7)	Herrlich	KFK
8)	Simons	Univ. Leiden

I. Summary of Project Global Objectives and Achievements

Ionizing radiation and UV-light induce a variety of reactions in mammalian cells, including mutations, DNA rearrangements, cell death and sometimes cancer. The mechanisms involved in the induction of the various responses are only partially understood. The purpose of this research proposal, in which 8 European laboratories collaborate, is to get an understanding of the responses that are induced in cells after exposure to ionizing radiation and UV-light.

The group of **Sarasin** investigates the nature and mechanism of induction of the mutations induced in human cells by UV-irradiation, using shuttle plasmids as probes. The mutations are compared with the mutations occurring in activated *ras* and p53 genes found in (UV-induced) skin tumors of Xeroderma pigmentosum (XP)-patients. These data may provide important information on the mechanism of radiation-induced carcinogenesis,

Three groups investigates the effects of UV- and ionizing radiation on gene expression. **Rommelaere's** laboratory focuses on the identification of radiation-induced DNA-binding proteins. As a probe the p4 promoter-enhancer of MVM parvovirus is used, which was found to contain a γ-ray-responsive element. Attempts are made to isolate DNA-binding proteins that are specifically induced or activated by γ-irradiation. Radiation-induced gene expression is also the main objective of **Herrlich's** laboratory. This group found that UV exposure of mammalian cells triggers signal transduction responses similar to those activated by treatment with growth factors. This culminates in the induction of growth-factor-responsive genes (e.g. c-*fos*, c-*jun*), and transient stabilization of p53. Ionizing radiation elicits responses that are in part different from those elicitated by UV, indicating that the reactions triggered by the two types of radiation are not identical. The laboratory of **Thomou-Politi** investigates the expression of the CD_2 antigen, a T-lymphocyte-specific surface antigen. Induction of CD_2 is particularly strong after low doses of X-rays, and it may serve as a sensitive indicator for exposure to ionizing radiation, particularly in the low dose ranges. The mechanism of enhanced CD_2 expression is currently under study.

The mechanism of induction of genetic instability by radiation and its relationship with mutation and cancer is the research subject of 2 laboratories. **Simons'** group

demonstrated that the ability to induce untargeted mutations (which is considered to be a manifestation of genetic instability) can be transmitted to unirradiated cells by incubation with conditioned medium from irradiated cells. It is likely that mitogens secreted by the cells in response to radiation (EPIF) constitute the active principle in the transmission.
The relationship between UV-induced genetic instability and skin cancer is the major research line of **Van der Eb's** laboratory. Fibroblasts from XP-patients that fail to develop skin cancer in spite of their strong UV-sensitivity, are defective in the UV-response Enhanced reactivation (ER). Attempts are made to identify the UV-induced responses that can explain this abnormal behaviour.

In line with the latter investigations are the studies of the laboratories of **Devoret** and **Bertazzoni**. Research by Devoret's group is focused on the role of the Kin17 gene in induction of genetic instability by radiation. Kin17 is a mouse gene, the product of which is possibly involved in (illegitimate) recombination. Expression of Kin17 in E.coli renders the cells sensitive to UV-light, suggesting it blocks a specific step in DNA repair, perhaps via a dominant-negative mechanism. Similar studies with mammalian cells are underway.
Finally, Bertazzoni's group investigates the biological and genetic characterization of UV-sensitive Trichothiodystrophy (TTD) and XP-group C fibroblasts. In the course of these studies the observation was made that the previously detected chromosomal rearrangements in an XP-group C fibroblast strain possibly reflect a precancerous state: in this cell strain an unusually high frequency of anchorage-independent growth was noted. The phenomenon might be a sign of genetic instability triggered earlier by (unrepaired) DNA damage. The group also investigates the role of poly(ADP-ribose)polymerase in the response to DNA damage.

Head of project 1: Prof. Dr. Van der Eb

II. Objectives for the reporting period

1. Characterization of UV-inducible phenomena in human cells

2. Radiation-induced carcinogenesis in transgenic mice

The objective of this investigation is to gain insight into the molecular nature of radiation-induced carcinogenesis. In project 1 the UV- or X-ray-induced responses are analysed in cell cultures derived from individuals which are resistant to (UV-induced) carcinogenesis or, in contrast, are highly susceptible to (spontaneous) cancer induction. Project 2 makes use of tumor-prone transgenic mice which carry either one activated oncogene or an inactivation of one of the two alleles of a tumor suppressor gene.

III. Progress achieved including publications

1. Characterization of UV-inducible phenomena in human cells

Exposure of human cells to UV or ionizing radiation results in the induction of a number of stress responses, including the Enhanced reactivation (ER) of UV-treated virus and Enhanced mutagenesis (EM) of untreated virus. Two of our recent observations have provided evidence that the ER phenomenon might be involved in, or co-regulated with, events leading to cancer induction. The evidence is (1) that ER is absent in cells derived from Xeroderma pigmentosum patients that did not develop skin cancer in sunlight-exposed areas as well as in cells derived from UV-sensitive but non-cancer-prone Trichothiodystrophy patients; (2) that a super-induction of ER occurs in cells derived from patients with hereditary cancer-prone syndromes. In fact, ER might serve as a prognostic marker for cancer-proneness in families affected by these disorders, which is currently under investigation. Work in the reporting period has primarily focused on the identification of the molecular mechanism of ER, or the mechanisms that are co-regulated with ER and might be aberrant in ER^- or ER^{super+} cells. The induction by UV of a number of UV-inducible genes is not affected in ER^- and ER^{super+} cells (e.g. c-jun, c-fos, metallothioneine, HSP70, collagenase, kin17). Induction of the ornithine decarboxylase

(ODC) however, is not observed in ER$^-$ XP-cells whereas only a very slight induction is found in ER$^-$ TTD-cells. Hence, UV-induction of ODC might be a property co-regulated with ER, which is currently under further investigation. UV-induced stabilization of the p53 protein was also studied in ER$^+$ and ER$^-$ cells. Although abnormal stabilization was found in XP-cells, and some ER$^-$ XP-strains failed to show UV-induced stabilization of p53, no consistent abnormality was observed associated specifically with the ER$^-$ phenotype. The abnormal kinetics in XP-cells in general is possibly caused by the fact that UV-damage persists much longer in XP-cells than in normal cells. In addition we have studied the UV-induced secretion of extracellular protein inducing factors (EPIF). Although we did find differences between the EPIF produced by ER$^-$ versus ER$^+$ cells, this difference was dependent on the acceptor cells that were used to test the EPIF and did not correlate with the ER$^-$ phenotype. Hence, the phenomenon will require further investigation.
Finally, we have tried to assess recombination/amplification rates in the ER$^-$ and ER$^+$ cells since these phenomena reflect genomic instability, which is considered to be an important factor contributing the carcinogenesis. These studies have been severely hampered by the fact that we are using non-immortalized skin fibroblasts which renders most traditional methods useless. To circumvent these problems we are currently trying to use SV40-transformed ER$^-$ and ER$^+$ cell lines.

2. Radiation induced carcinogenesis in transgenic mice

As reported previously, our attempts to use cells derived from transgenic mice for in vitro transformation studies were unsuccessful. Hence, we have shifted our attention to radiation-induced tumor formation in transgenic mice. In collaboration with Drs. Van Steeg and Van Kreijl (Bilthoven) we have characterized tumors induced with benzo[a]pyrene in Eμ-pim1-transgenic mice. The majority of tumors are T-cell lymphomas. In contrast to earlier studies with these Eμ-pim1 mice treated with different agents, we now observed only a few cases of c-myc amplification. We are now attempting to identify which (other) oncogenes have been activated in these tumors. We will soon start tumor induction experiments with both ionizing or UV-irradiation, using transgenic mice (Eμ-pim1 and Rb-knock-out).

Publications

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Geert Weeda, Libin Ma, Reinier C. van Ham, Alex J. van der Eb and Jan H.J. Hoeijmakers. Structure and expression of the human XPBC/ERCC-3 gene involved in DNA repair disorders xeroderma pigmentosum and Cockayne's syndrome. Nucl.Acids Res. 19(1991)6301-6308.

Geert Weeda, Libin Ma, Reinier C.A. van Ham, Dirk Bootsma, Alex J. van der Eb and Jan H.J. Hoeijmakers. Characterization of the mouse homolog of the XPBC/ERCC-3 gene implicated in xeroderma pigmentosum and Cockayne's syndrome. Carcinogenesis 12(1991)2361-2368.

Libin Ma, Geert Weeda, Aart G. Jochemsen, Dirk Bootsma, Jan H.J. Hoeijmakers and Alex J. van der Eb. Molecular and functional analysis of the XPBC/ERCC-3 promoter: transcription activity is dependent on the integrity of an Sp1-binding site. Nucl.Acids Res. 20(1992)217-224.

Bernd Stein, Peter Angel, Hans van Dam, Helmut Ponta, Peter Herrlich, Alex van der Eb and Hans Rahmsdorf. Ultraviolet light induced c-jun gene transcription: two AP-1-like binding sites mediate the response. Photochem. and Photobiol. 55: 409-415, 1992.

P.J. Abrahams, A. Houweling and A.J. van der Eb
High levels of Enhanced reactivation of HSV-1 in UV-treated skin fibroblasts from various hereditary cancer-prone syndromes. Cancer Res. 52: 53-57, 1992.

Hans van Dam, Monique Duyndam, Robert Rottier, Anne Bosch, Lydia de Vries-Smits, Peter Herrlich, Alt Zantema, Peter Angel and Alex J. van der Eb. Heterodimer formation of cJun and ATF-2 is responsible for induction of c-jun by the 243 amino acid adenovirus E1A protein. EMBO J. 12 (1993)479-487.

Head of project 2: Dr. Sarasin

II. Objectives for the reporting period

1 - Analysis of mutagenesis at the molecular level using shuttle vector.

- Description of a new shuttle vector
- Validation of the lac Z' as a target for mutagenesis

2 - Gene modifications in UV-induced human skin tumors

- ras mutation and amplification
- mutation on the P53 tumor suppressor gene.

Objectives for the next reporting period

1 - Analysis of UV-induced mutation spectra in cultured human cells from normal or DNA repair-deficient individuals.

2. P53 mutations in UV-induced human skin tumors.

III. Progress achieved including publications

1. Analysis of mutagenesis at the molecular level using shuttle vector :

Most of the experiments using shuttle vectors in human cells are carried out with the pZ189 plasmid which is based on the supF tRNA gene. This target is very interesting but the putative presence of secondary structures and eventually of single-stranded areas renders it difficult to consider this sequence as a "classical" cellular gene. Since part of our projects deals with the analysis of mutation spectra in mammalian cells subjected to various treatment we develop a new shuttle vector based on a mutagenesis target rarely used in higher eukaryotes : the lac Z' gene. This gene represents about 150 base pairs and can be screened easily in bacteria by complementation on the lac operon. The absence of mutation on the plasmid leads to blue bacterial colonies while a mutation changing the amino-acid leads to a white colony.

We have constructed the pR2 plasmid where the lac Z' gene is located between the promoter and the coding sequence of the kanamycin resistance gene, used for bacterial selection. This target is very stable and a spontaneous mutation frequency of about 10^{-4} is observed in several transformed human cells. In order to validate the pR2 vector for mutagenesis studies we irradiated it with UV-light (254 nm) at

various fluences from O to 1000 J/m^2. The mutation frequency can be increased by a factor greater than 70. The majority of mutations (> 90 %) are point mutations located at dipyrimidine sites. The substitution hot spots are at C-C sequences leading to C to T transitions, as already observed with other model systems.

The pR2 shuttle vector has proven to be a good assay for mutagenesis studies in human cells. Its low spontaneous mutation frequency and its new target render it a very nice probe for our future studies.

2. Gene modifications in UV-induced human skin tumors :

Xeroderma pigmentosum (XP) patients are clinically characterized by a very high incidence of skin cancers on exposed skin, at an early age. XP cells in vitro are strongly deficient in excision-repair and highly mutagenized by UV-light. We were, therefore, interested in measuring mutation frequency and in determining mutation spectra in patients' tumors exposed to UV lesions. We chose to look at oncogene activation in skin tumors with the idea that more mutations, particularly of the ras gene family, would be found in XP tumors where lesions remain unrepaired compared to normal individuals. Our results clearly show that more than a 2-fold significantly higher mutation frequency (50 %) of the ras genes was found in XP in contrast to control tumors (22 %).

The majority of the mutations were found at codon 12 of all three ras genes with a preponderance for N-ras in XP samples. The mutation spectra indicate that all mutations found were located opposite pyrimidine-pyrimidine sequences which represent a hot spot for UV-induced DNA lesions. Most of the mutations were of the type expected from studies performed in vitro with model systems. This high mutation frequency in XP was accompanied by a very high level of Ha-ras and c-myc gene amplification and rearrangement. All these data are consistent with a fundamental role of unrepaired UV-induced DNA lesions as an initiating event in human skin tumors on exposed parts of the body.

The p53 gene is frequently mutated in human cancers and represents a good target for studying mutation spectra since there are more than a hundred potential sites for phenotypic mutations. Using reverse transcription PCR and single strand conformation polymorphism to analyse over 40 XP skin tumors (mainly basal and squamous cell carcinomas), we have found 40 % (17/43) contained at least one point mutation on the p53 gene. Indeed 4 XP tumors contained point mutations on the two alleles of the P53 gene. All the mutations were located at dipyrimidine sites, essentially at C-C sequences, which are hot spots for UV-induced DNA lesions. 61 % of these mutations were tandem CC to TT mutations considered to be unique to UV-induced lesions and are not observed in internal human tumors. All the mutations, except two, must be due to translesion synthesis of unrepaired dipyrimidine lesions left on the non-transcribed strands. These results show for the first time the existence of preferential repair of UV lesions (either pyrimidine dimers or pyrimidine (6-4) pyrimidones) on the transcribed strand in human tissues.

Interestingly, we can compare mutation spectra in DNA repair deficient patients by using either cultured cells replicating shuttle vectors or directly tumor biopsies. Although the two biological materials are different the obtained results are very similar and complementary while studying mutation characteristics.

Head of project 3: Dr. Devoret

II. Objectives for the reporting period

Seeking to isolate from the mouse a gene encoding a protein involved in SOS functions, we discovered on mouse chromosome 2, band A, a new gene, we called KIN17, since it encodes an amino acid region *akin* to that of RecA protein, which controls procaryotic SOS functions. Our objectives were: (1) to sequence the full KIN17 cDNA and find amino acid regions of interest; (2) to clone KIN17 cDNA into a suitable vector to express high amounts of kin17 protein in *E. coli*; (3) to purify kin17 protein and determine whether it interacts with DNA; (4) to establish a role for kin17 in mammalian cells; (5) to clone the full mouse or human KIN17 gene. As seen in the progress report below, most of these objectives were met.

Our objectives for the next period are based on the predictable structure of KIN17 whose nucleotide sequence displays three *putative* regions of interest: a zinc finger, a nuclear localization signal, a region of homology with *E. coli* RecA protein.

We aim at determining the functional role of these domains in kin17 binding to DNA, namely, (1) the functionality of the kin17 zinc finger; (2) the functionality of the nuclear localization signal in driving kin17 protein to the cell nucleus; (3) the involvement of the RecA protein homologous region in DNA binding; (5) the nature of kin17 DNA binding - random or preferential to some DNA specific sequences or DNA structures; (6) the involvement of kin17 in homologous or non-homologous recombination and in repair.

III. Progress achieved including publications

Cloning a mouse cDNA expressing a protein cross-reacting with anti-RecA antibodies

Antibodies have been used to detect proteins sharing structural similarities. The immunological detection of epitopes coupled with recombinant DNA technology has provided a potent tool for cloning new genes encoding selected polypeptides.

By cytological methods we had detected a *nuclear* protein cross-reacting with anti-RecA antibodies in *actively dividing cells* of mouse embryos, we set to clone its cDNA and identify a protein possibly involved in mammalian SOS functions.

We went through two successive steps: (1) from embryonic mRNA we obtained a partial cDNA fragment, $KIN17_{601}$; this fragment was used as a probe to clone the full *KIN17* cDNA.

***KIN17* gene was localized by cytogenetic mapping to mouse chromosome 2, band A and to the short segment of human chromosome 10**

Homologs to *KIN17* cDNA were detected in rat and human DNAs. In the human genome the *KIN17* gene sequence is on the short arm of chromosome 10. The vimentin gene is the closest marker to *KIN17* gene in mouse and human chromosomes indicating a synteny between the murine 2A region and the human 10p region. Nucleotide sequences

gene is the closest marker to *KIN17* gene in mouse and human chromosomes indicating a synteny between the murine 2A region and the human 10p region. Nucleotide sequences of the *KIN17* gene are conserved in the three DNAs of the mouse, rat and man.

KIN17 DNA encodes a zinc finger, a nuclear localization signal and a region homologous to RecA protein

KIN17 cDNA is 1414 bp long with an open reading frame of 391 amino acids. Computer analysis shows three putative regions of interest: (1) a zinc finger, (2) a nuclear localization signal, and (3) a region of homology with prokaryotic RecA protein that spans over 39 amino acids.

1. *Zinc finger.* The zinc-binding domain, between residues 28 and 50, is of the $CX_2CX_{12}HX_5H$ type, belonging to the Cys-Cys...His-His zinc-finger protein subclass. Kin17 has only one zinc finger unit like proteins of the Cys-Cys...Cys-Cys subclass. Interestingly, in the kin17 zinc finger there are 2 cysteines flanking the first histidine residue at the C-terminal part of the motif that might be involved in a Cys-Cys...Cys-Cys type of coordination complex.

2. *Nuclear localization signals.* In mouse embryos, the material reactive with anti-RecA antibodies is in the nucleus. In collaboration with V. Schreiber, J. Menissier and G. de Murcia (IBMC, Strasbourg), we have demonstrated that kin17 residues 234 to 264 are bona fide nuclear localization signals.

3. *Region homologous to RecA protein.* This is a region large enough to define an antigenic determinant. It may also be a motif governing protein binding to DNA.

4. *kin17 homology to poly-ADP-ribose polymerase.* There is substantial homology between kin17 and mouse poly-ADP-ribose polymerase, suggesting that kin17 may also be involved in DNA repair.

KIN17 mRNA, found in neural cells, is increased after X-irradiation

KIN17 mRNA was shown to be expressed in neural rodent cells. It is induced by irradiation with X-rays (J.F. Riou, Rhone-Poulenc).

Purified kin17 protein binds to single-stranded DNA and to double-stranded DNA

We have produced kin17 protein in *E. coli*, purified it up to more than 90%. The observed M_r *E. coli*-produced kin17 protein is 43 kDa and agrees with the size of the observed mRNA in mammalian cells. We have demonstrated that kin17 binds to single-stranded DNA and to double-stranded DNA.

It remains to be established whether the DNA-binding activity of Kin17 is mediated by the zinc-finger, or by the RecA protein antigenic motif or by both regions.

Conclusions

Numerous cases of similarity between mammalian, yeast and bacterial proteins involved in DNA repair have been reported. For instance, the human genes *ERCC1* and *ERCC2* are analogs of *RAD10*, *RAD3* excision repair yeast genes as well as *E. coli uvrC* and *uvrA*. It was shown very recently that RecA protein involved in recombination repair and mutagenesis in procaryotes has homologs in yeast, in chicken and human cells.

Here is a summary of our contribution to the fast-evolving field. We have discovered a gene that we have called KIN17, 1414 bp long, located on mouse chromosome 2, band A. We found genomic sequences homologous to *KIN17* cDNA in rat and human

DNAs. KIN17 gene encodes a newly identified mammalian nuclear protein, 391 amino acid long, with three domains of interest: a zinc finger, a nuclear localization signal, a region homologous to RecA protein. Kin17 shares a substantial homology to poly-ADP-ribose polymerase, which appears to be involved in DNA repair. Purified kin17 protein binds to single-stranded and to double-stranded DNA. Kin17 protein is highly expressed in neural cells.

Laboratory publications for the last 3 years

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Head of project 4: Prof. Rommelaere

II. Objectives for the reporting period

(i) One of the major objectives for the reporting period was the identification of cellular DNA-binding proteins induced by ionizing radiation, using parvoviral DNA as a probe. In this framework, we prepared a series of linker-scan mutations in the promoter enhancer region of plasmid pP4-Luc, which carries the firefly luciferase gene under control of the pivotal MVM P4 promoter. These promoter mutants will facilitate the identification of promoter elements involved in the cellular responses to ionizing radiation.

(ii) Furthermore, this project aimed at constructing parvovirus-based vectors to be used in the generation of transgenic mice expressing stress-inducible parvoviral genes with oncosuppressive properties. As a first step to analyse the control of parvovirus gene expression *in vivo*, transgenic mice were generated, carrying the β-galactosidase gene under control of the MVMp P4 promoter.

(i) The first objective for the next reporting period consists in the identification and characterization of ionizing radiation-induced cellular DNA binding proteins. If need be, corresponding gene clones and antisera will be prepared. The above-mentioned linker-scan mutants will be used to determine DNA-responsive elements that serve as targets for the radiation-induced modulation of parvovirus growth, in particular for the previously reported enhanced capacity of irradiated cells to replicate and express parvoviral DNA. The possibility that some of the cellular factors identified in this way may control both DNA amplification and transcription will be explored.

(ii) The production of transgenic mice carrying the parvoviral non-structural genes under control of their genuine P4 promoter, will be attempted. If viable, these animals will be tested for the resistance to tumor induction by radiations and other treatments.

III. Progress achieved including publications

I Cellular level.

The identification of radiation-induced cellular factors, using parvoviral DNA sequences as probes, was one of the main goals for the reporting period. The P4 promoter enhancer region of parvovirus MVMp includes both (a) putative stress response element(s) [mediating replication signals elicited by radiation or chemical carcinogens] and the palindromic origin of viral DNA amplification. A large series of overlapping oligonucleotide duplexes from the promoter region were used in electrophoretic mobility shift assays (EMSA's). Differential gel-shift patterns were obtained between normal and γ-ray transformed human fibroblasts using an oligonucleotide which carries an inverted CCAAT box overlapping a sequence that is closely related to the known binding site of a γ-ray inducible cellular factor (see below). Similar differential gel shift patterns were obtained with immortalized but otherwise normal rat fibroblasts and v-*ras* or Ha-*ras* transformed derivatives.

Sing and Lavin have recently identified a sequence from the SV40 early promoter enhancer that binds a γ-ray induced nuclear factor(s). This factor is normally restricted to the cytoplasm, but is rapidly translocated to the nucleus following cell irradiation. Most interestingly, cells from patients suffering from the radiosensitive syndrome Ataxia telangiectasia constitutively express this cellular factor in the nucleus. The relevant MVM and SV40 oligonucleotides display a strong homology, suggesting that the former could also bind this factor. Experiments to test this possibility are in progress.

In addition, the MVM oligonucleotide that produces a differential gel shift between normal and radiotransformed cells, carries an inverted CCAAT box. Several CCAAT box-binding factors have been identified and appear to belong to distinct families of transcription factors with overlapping and often poorly characterized binding properties. Antibodies directed against the A or B chain of the CCAAT box-binding protein N-Y were found to cause supershifts in EMSA's performed with the

MVMp oligonucleotide, suggesting that the latter harbours an NF-Y binding site. Yet, the MVMp CCAAT box is not identical to the reported consensus NF-Y responsive element. Conversion of the MVM sequence to a consensus core NF-Y motif increased binding by approximately 2 to 3 - fold. Thus, the MVM-derived oligonucleotide appears to contain a low affinity but genuine NF-Y binding site. NF-Y is an ubiquitous nuclear protein composed of two unrelated subunits (A and B) that are both required for DNA binding. This factor controls the transcription of target genes (e.g. albumin and MHC class II) by binding to a Y-box enhancer sequence. Most autonomous parvoviruses share with MVMp the presence of a similar NF-Y binding site at the same position in the genome, suggesting that this factor may be essential for virus transcription, replication or both. That NF-Y may play a role in the transduction of signals generated by oncogenic transformation is supported by the recently reported involvement of a factor with similar DNA-binding properties in the modulation of a retroviral long terminal repeat by *src*-induced transformation. Altogether, these date prompt us to consider further testing whether the differential gel-shift pattern observed with the MVM oligonucleotide is due to a distinct post-transcriptional modification of the NF-Y protein in transformed versus normal cells.

II Animal level.

Part of our efforts during the reporting period was devoted to the construction of plasmids that carry the parvoviral non structural (NS) genes and will be used for the generation of transgenic mice. It was previously shown that expression of the NS-1 polypeptide is cytotoxic, while the smaller non-structural protein NS-2 enhances the NS-1-induced cytopathic effect in SV40 transformed human cells (Caillet-Fauquet et al., EMBO-J, 9, 2989-2995, 1990). In contrast, several lines of evidence suggest that the NS proteins are not or less toxic for normal cells. The lytic effect of NS polypeptides on oncogene-transformed cells, together with the relative innocuousness of these products for normal cells, form the basis of our project that aims at producing transgenic mice harbouring NS genes under control of their genuine P4 promoter, at determining whether these mice are protected against x-ray induced tumorigenesis.

The P4 promoter and NS-coding sequence were cloned in the pAT153 plasmid (thus deleting the long non-translated 3' portion of NS-encoding viral mRNAs) and correct termination and processing were ensured by insertion of the SV40 small t antigen splice and termination sites. The recombinant plasmid produced biologically active NS-1 protein as deduced from its ability to transactivate the second (P38) MVM promoter in transient expression assays. A similar construction, in which the β-galactosidase reporter gene was placed under the control of P4, was also obtained and used, in a preliminary step, to investigate the tissue distribution of P4 activity in transgenic mice. Galactosidase expression was detected consistently in the cerebellum and occasionally in the eyes of 12 day-old mouse embryo's. As brain cells do not actively divide at this stage of development, no dramatic cytotoxicity is expected in transgenic mice due to the targeted expression of NS proteins during normal ontogenesis.

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Head of project 5: Dr. Bertazzoni

II. Objectives for the reporting period

Cellular and genetic characterization of trichothiodistrophy (TTD) and xeroderma pigmentosum (XP) patients. Chromosomal instability in homozygous and heterozygous carriers of XP mutations. Cellular and genetic characterization of mutagen-sensitive rodent mutants (F. Nuzzo and M. Stefanini).

Study of protein acceptors modified by poly(ADP-ribose)polymerase (ADPRP) in mammalian cells treated with mutagens. Analysis of expression and stability of mRNA for ADPRP. Study of the distribution of antibodies to ADPRP naturally occurring in autoimmune diseases (U. Bertazzoni and A.I. Scovassi).

Objectives for the next reporting period

Chromosomal instability, cellular transformation and cancer proneness in patients affected by xeroderma pigmentosum (XP). Study of the association of trichothiodystrophy (TTD) with DNA repair defects. Cellular and genetic characterization of mutagen-sensitive rodent mutants.

Analysis of the effect on ADPRP activity of the treatment of human cells with antitumor drugs acting as inhibitors of DNA topoisomerases II. Study of the role of post-translational modification of proteins such as ADP-ribosylation in the regulation of programmed cell death. Study of the correlation between clinical features of autoimmune diseases and appearance of autoantibodies to ADPRP.

III. Progress achieved including publications

Cellular and genetic characterization of DNA repair mutants

DNA repair investigations in patients affected by trichothiodystrophy (TTD), a rare autosomal recessive disease characterized by brittle hair with reduced sulfur content, mental and physical retardation, peculiar facies, ichthyosis and, in many cases, photosensitivity, have demonstrated that the photosensitivity correlates with an altered cellular response to UV damage. Fifteen repair defective TTD cell strains showing different degrees of repair alterations were assigned to xeroderma pigmentosum complementation group D (XP-D). Two further defective TTD strains (referred to us by dr. Lehmann - MRC Cell Mutation Unit, Falmer, U.K., and by dr. A. Sarasin -IRCS, Villejuif, Fr.) appeared to be able to complement each other and in neither case did they fall into the XP-D group. Furthermore in one of these strains, the defect was different from those present in all the XP excision-repair defective groups. This cell strain may therefore represents a new human gene involved in DNA repair.

To investigate the possibility that the clonal chromosome rearrangements previously detected in a XP group C fibroblast strain (XP9PV) reflected the presence in the population of cells at an early stage of tumor development, the anchorage independet (AI) growth, a characteristic strictly linked to cellular transformation, was analyzed in XP9PV cells. A higher frequency of AI clones was detected in XP9PV

(3.2×10^{-2}) compared to a control fibroblast strain (1.3×10^{-4}). This result confirms the genomic instability and proneness to mutation of the XP9PV strain already detected at karyotypic level. 26 colonies growing in agar were isolated and propagated and all of them show a normal fibroblast morphology. The cytogenetic analysis of these clones is under way.

Genetic characterization of mutagen-sensitive clones isolated from rodent cell lines led to the identification of a new complementation group of UV-sensitive rodent cell lines, the eleventh group. By complementation analysis we demonstrated that the repair defect in the mutant UVS1 (isolated from the Chinese hamster cell line CHO9 and previously found to complement the UV sensitivity of mutants representative of groups 1 to 7) was genetically different from those present in mutants belonging to groups 8, 9 and 10.

Study of the role of poly(ADP-ribose)polymerase in mammalian cells

The characterization of non-histone proteins ADP-ribosylated in human cells, focused on the 170 kDa peptide ADP-ribosylated in physiological conditions in HeLa cells, provided the evidence that this protein corresponds to DNA topoisomerase II. Our results demonstrated that topoisomerase II is modified in vivo by the enzyme poly(ADP-ribose)polymerase. In HeLa cells treated with dimethylsulfate the modification of DNA topoisomerase II is not affected by the damage to DNA thus suggesting that the modification of the enzyme is not regulated by DNA repair.

The reaction catalysed by ADPRP is strictly dependent on the presence of DNA strand breaks and causes a consumption of NAD; as a result of massive autoribosylation both NAD and ATP levels fall very rapidly and this event could mediate the suicide response of cells severely damaged. To determine whether this effect can be counterbalanced by divalent ions, we have treated HeLa cells with dimethylsulfate in the presence of Zn^{2+}. The activity of ADPRP, which usually decreases dramatically following treatment with high concentrations of drugs, is preserved by the administration of Zn to the cells, indicating that this ion blocks the formation of DNA strand breaks.

We have studied the regulation of the expression of the ADPRP gene in HeLa cells and quiescent and mitogen-stimulated human lymphocytes. The analysis consisted of the quantitation of mRNA molecules by means of a new technique based on PCR and involves co-amplification of a competitive target template by using the same primers as those of the cDNA targer obtained by reverse transcription of the mRNA species. The results obtained comparing quiescent and proliferating lymphocytes or HeLa cells indicate that the proliferation state induces a notable increase in the expression of the enzyme. To determine the stability of mRNA for ADPRP, a new PCR amplification system was developed, using non-competitive conditions. We observed that in quiescent cells the half-life of ADPRP mRNA is about 1 h, whereas in proliferating cell cultures it was calculated to be about 4-5 fold higher.

The search for autoantibodies to ADPRP in sera from human patients affected by autoimmune diseases confirmed that this enzyme has to be considered as a new target of autoimmunity. Preliminary results indicate that the presence of specific autoantibodies could be correlated to the pulmonary involvement of some diseases.

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Head of project 6: Dr. Thomou-Politi

II. Objectives for the reporting period

a) The modulation of CD2 expression in X-irradiated CHO cells transfected with πH3 vector carring the human CD2-cDNA, has been investigated. We have studied the effect of X-rays on the level of CD2 protein expression.
b) The effects of ionizing radiation on the CD2 gene expression in normal human T lymphocytes, has been investigated.

Objectives of the next reporting period

The effects of ionizing radiation on the regulation of gene and protein expression is pleiotrophic. Some genes are upregulated by radiation, while others are unaffected or down regulated.

The CD2 gene is constitutively expressed in resting T cells and its transcription is over-induced after PHA treatment. We will focus on CD2 gene expression in normal human T lymphocytes in order to distinguish between transcriptional and post transcriptional effects of X-rays.

Thus, we are going to study if the accumulation of CD2 mRNAs after X-irradiation (0-100 cGy) of resting and PHA treated T lymphocytes is due to either stimulation of transcription and/or stabilization of preformed mRNAs.

III. Progress achieved including publications

Introduction

It is well known that the effects of ionizing radiation on the regulation of gene and protein expression is pleiotrophic. Some genes are upregulated by radiation while others are unaffected or down regulated.

The CD2 gene encodes a cell surface T-cell specific antigen that appears early in thymic ontogeny and is involved in cell-cell adhesion, signal transduction, T cell activation, differentiation and immune response. This Ag is expressed in thymocytes, T lymphocytes and NK cells and also is the receptor of sheep red blood cells (SRBC). We have thus, examined the effects of very low doses of X-irradiation on the expression and function of the CD2 surface antigen in $CD2^+$ CHO cells and in resting and PHA activated human T lymphocytes.

Results

1) Effects of X-ray irradiation on the CD2 expression in $CD2^+$ CHO cells.

Thioguanine resistant CHO cells were stably cotransfected with πH3-CD2 and pSV2-gpt vectors. The resulted cotransfected CHO clones are HAT resistant and constitutively express the human cell surface CD2 antigen as was verified by their ability to form rosettes with sheep red blood cells (SRBC). Moreover when $CD2^+$ CHO clones were incubated with anti-CD2 mAb (OKT11), stained with FITC and analyzed by flow cytometry revealed a pattern

of fluorescence intensity similar to that of peripheral T lymphocytes. One clone namely CL13, the most enriched in rosette positively cells, was chosen for further study.
Southern blot analysis of genomic DNA cleaved with various restriction endonucleases revealed that the $CD2^+$ CL13 clone contains only one copy of πH3-CD2 vector integrated in the CHO genone.
The C13 cells were further tested by titrating their bioresponse to low doses of radiation affecting the quantitative expression of the CD2 antigen. It was found that very low doses of X-ray (2-6 cGy) did not affect the level of CD2 antigen compared to unirradiated control as was verified by rosette assay and flow cytometric analysis. In contrast, the irradiated CL13 cells seemed to be very sensitive at 10-50 cGy showing decreased levels of fluoresence which could be attributed to the fact that around 50% of the cells have lost the $CD2^+$ phenotype and/or the CD2 expression was deduced in all $CD2^+$ cells.
Southern blot analysis of genomic DNAs from CL13 cells given 0-10 cGy of X-irradiation showed that no extra or missing bands and no amplification have occured compared to unirradiated control.

Although the explanation for the mechanism involved in the loss of $CD2^+$ phenotype is obscure a number of possibilities may be considered. It is known that cell membrane and receptors may act as primary target for low doses of irradiation. So, the partial inhibition of erythrocyte rosette formation that we report may be attributed to the fact that free vadicals produced by ionising radiation may affect the conformation of the CD2 receptor leading to a partial loss of $CD2^+$ phenotype in CL13 cells. Alternatively, a tentative hypothesis is to consider that low doses of X-irradiation trigger a trans-activating signal which swithes off CD2 expression.

2) Effects of ionizing radiation on the CD2 gene expression in normal human T lymphocytes

Human peripheral blood lymphocytes were ficoll isolated, irradiated at doses from 0-100 cGy and cultured for 24 and 48 hours with or without suboptimal concentration of PHA, which as itis known over-induces the transcription of the CD2 gene and activates human T-cells.
At the end of the the culture they analyzed a) for their ability to form rosettes with SRBC and b) by flow cytometry using anti-CD2 mAb (OKT11).

The results have shown that resting and PHA activated T lymphocytes bind more SRBC on their cell surface and also reveal a transposition to higher levels of fluoresence intensity when very low doses up to 50 cGy of X-irradiation were given. In contrast irradiated T cells with 100 cGy behave almost similarly to the unirradiated control.

Moreover the level of CD2 mRNAs was significantly higher in X-irradiated T lymphocytes than in control cultures as it was measured by Northern blotting.
Whether the accumulation of these mRNAs is the concequence of either stimulation of transcription, stabilization of preformed mRNAs or both remains to be elucidated.

Publications

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Head of project 7: Dr. Herrlich

II. Objectives for the reporting period

Various radiation qualities such as ultraviolet irradiation, X-rays or alpha-rays induce similar cellular reactions, as e.g. cell killing, DNA repair, a stop of DNA synthesis and mutagenesis. Most if not all cellular reactions to UV-irradiation seem to be mediated by proteins, whose synthesis or activity is modulated after UV irradiation: affected proteins include the transcription factors c-Jun, Jun B and c-Fos, and several growth factors. UV irradiation modulates the activity of these proteins by initiating a signal transduction chain. In the reporting period we analyzed the signal transduction pathways initiated by X-rays and compared them to signal pathways initiated by UV and by tumor promoter phorbol esters. We focused on the question where the signal pathways originate from and which proteins that are activated in the signalling process, are essential members of the signal chains. Flow of signals was measured as posttranslational activation of the mitogen activated protein kinase, as posttranslational activation of the transcription factors Fos and Jun, by the transcription of the early genes c-fos and c-jun and by the stabilization of the nuclear oncoprotein p53.

Objectives of the next reporting period: We wish analyse in molecular terms X-ray and UV induced gene expression and p53 stabilization. Major emphasis will be on steps preceding X-ray induced activation of a cytoplasmic protein kinase (mitogen activated protein kinase) and on similarities and differences of the UV and X-ray induced stabilization of the nuclear oncoprotein p53. The possibility will be explored whether DNA repair is an intermediate in p53 stabilization.

III. Progress achieved including publications

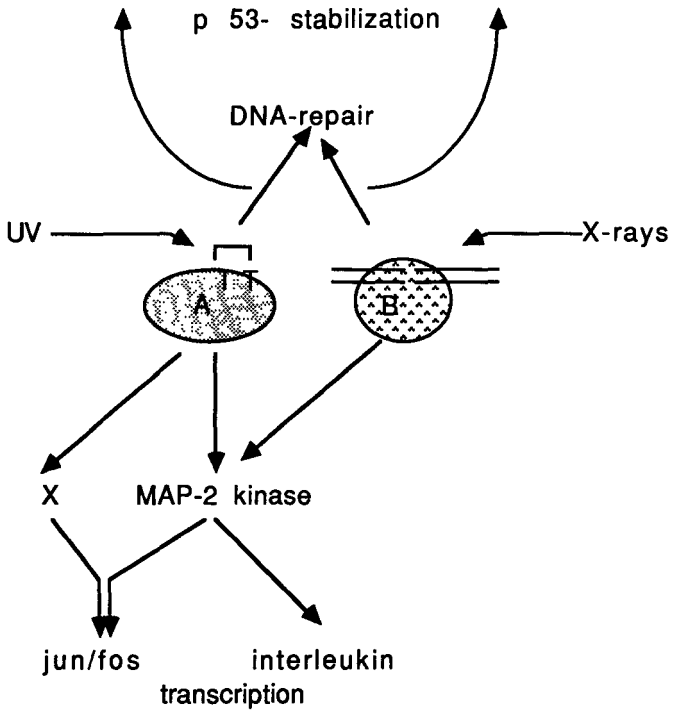
UV irradiation of cells in culture induces the activation of transcription factors, the secretion of growth factors and the increased transcription of genes coding e.g. for the transcription factors c-Fos, c-Jun and Jun B, and for secreted proteases such as collagenase I and plasminogen activator. Immediately after UV irradiation the cell membrane associated tyrosine kinases src and fyn are activated, Ras is converted to its GTP-binding form and the cytoplasmic protein kinases raf and Map-2 (mitogen activated protein kinase) are activated (Devary et all, Cell 71, 1081, 1992; Radler-Pohl et al, EMBO J. 12, 1005,1993). We have investigated whether the UV induced cellular reactions are essential members of the signal chain leading to enhanced gene transcription, and whether the UV induced signal chain originates from damage to DNA. We could demonstrate that the UV induced secretion of interleukin 1 alpha and basic fibroblast growth factor from HeLa cells is a necessary intermediate in UV induced collagenase gene expression; this is suggested by our finding, that suramin (an inhibitor of growth factor/growth factor receptor interactions) interferes specifically with UV induced collagenase mRNA accumulation and that antibodies directed against interleukin 1 alpha and basic fibroblast growth factor also interfere with this process (Krämer et al, J. Biol Chem. 268, 6734,1993). Suramin also interferes with the very early reactions after UV such as raf and MAP-2 kinase activation, suggesting that also these early reactions

involve the activation of membrane associated growth factor receptors (Radler-Pohl and Sachsenmaier, unpublished). UV induced ras and raf activation are obligatory steps in the UV-induced signal chain (Devary et al,Cell 71,1081, 1992; Radler-Pohl et al, EMBO J. 12, 1005, 1993; Sachsenmaier, unpublished). Concerning the question whether the UV induced signal leading to the activation of cytoplasmic protein kinases stems from the nucleus, we could demonstrate, that in contrast to intact cells, cytoplasts (from which the nucleus had been expelled by cytochalasin B treatment of cells) and platelets (which normally do not possess a nucleus) do not react to UV irradiation by the activation of MAP-2 kinase, while they do react to treatment with phorbol esters or growh factors (Radler-Pohl, unpublished).

In contrast to UV, X-rays at a dose of 500 or 5000 rad induce fos and jun RNA accumulation much less efficiently in HeLa cells. X-rays do, however, induce MAP-2 kinase activation, to an extent and with kinetics similar to those seen with UV. This suggests that either UV-induced MAP-2 kinase activation is only one of several parallel steps, all of which are induced by UV (but not by X-rays) and are needed for UV-induced transcription of c-fos and c-jun; or that X-rays, in contrast to UV, induce an inhibitory step, which disconnects activation of MAP-2 kinase from downstream targets (the transcription factors leading to the transcription of c-fos and c-jun). The transcription of other genes such as the gene coding for interleukin 1 alpha is induced with similar efficiency by UV- and by X-rays. These results suggest that different proteins perceive UV and X-ray induced signals and that these proteins feed signals into pathways which are common to UV and X-rays and into pathways which differ beween the two radiation qualities. As with UV, also X-ray induced gene transcription does not depend on the repair of X-ray induced DNA damage.

This situation is different for another radiation induced process, namely the stabilization of the nuclear oncoprotein p53. Radiation induced p53 stabilization occurs in a time frame of 15 minutes to two hours and after low doses of UV or X-rays (down to 20 rads; Maltzman and Czyzyk, Mol Cell. Biol. 4, 1689,1984; Kastan et al, Cell 71,587,1992 ; Blattner, unpublished). X-ray induced p53 stabilization occurs in wild type cells and in Xeroderma pigmentosum cells (which can repair X-ray induced DNA damage), but not in Ataxia telangiectasia cells (which cannot repair X-ray induced DNA damage; Kastan et al, Cell 71, 587, 1992; Blattner, unpublished). UV induced p53 stabilization occurs in wild type cells and in all Ataxia teleangiectasia cells (which can repair UV induced DNA damage), but not in Xeroderma pigmentosum cells (groups A trough E), which cannot repair (Blattner, unpublished). These findings show a strict correlation of the capacity of a cell to deal with DNA damage and the stabilization of p53, suggesting that the process of DNA repair or a byproduct of it leads to the stabilization of p53.

Our findings may be summarized in the following working hypothesis:



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Head of project 8: Dr. Simons

II. Objectives for the reporting period

Previously it has been found that DNA damage leads, next to the direct induction of mutations, to untargeted mutations due to an infidelity of DNA replication in the progeny of treated cells. This phenomenon has been ascribed to the induction of a generalized stress response in all the treated cells which is transmitted to the progeny. If this interpretation is correct it should be possible to induce genetic instability in cells by treatment with EPIF. EPIF (Extracellular Protein synthesis Inducing Factors), is secreted by UV-irradiated cells and switches on a stress response in undamaged cells. This has been investigated. Another objective was to determine whether ionizing radiation also leads to the induction of genetic instability.

Objectives for the next reporting period
Because the background of the spontaneous mutation rate could be a determining factor in the degree of induced genetic instability it will be investigated whether this rate is variable and whether it can be experimentally manipulated.
Another objective relates to the fact that induction of a stress response in mammalian cells in vivo is involved in phenomena like inflammation and wound healing. Wound healing involves among others that cells acquire the capacity to migrate. Therefore it will be pursued to develop an assay system with mouse keratinocytes which can measure the degree of induction of migration competence. Such an assay system will allow to identify morphologically the induction of a stress response in these cells and to establish whether the alteration in phenotype leads to genetic instability and/or malignant cell transformation.

III. Progress achieved including publications

Induction of genetic instability by stress-induced secreted proteins.
To test whether induction of stress responsive proteins are involved in the induction of genetic instability mouse T lymphoma cells, GRSL13, were treated with EPIF (Extracellular Protein synthesis Inducing Factors). For the production of EPIF, growing or stationary cells were irradiated with 15 J/m^2 UV and incubated for 2 days. Medium from irradiated cells (EPIF medium) was collected and transferred to either growing or stationary cells. After 1 day incubation in EPIF medium, 25000 viable cells were expanded to about 10^8 cells in normal medium and the mutation rate was determined. Using different conditions for treatment and culture in 48 determinations showed that overall EPIF significantly enhanced the mutation rate 1.8-fold ($P=0.008$). EPIF only enhanced the mutation rate when the EPIF producing cells and the EPIF treated cells were in the same condition, either both growing or both stationary. This indicates that the EPIF from growing and stationary cells is different and that the growing and stationary cells differ in their sensitivity to the excreted factors.
As the spontaneous and the EPIF induced mutation rate could be influenced by factors in the serum, EPIF was also produced in serum free medium supplemented with BSA. Stationary cells were used both as producer cells and target cells. A 2.8-fold enhancement

of the mutation rate after treatment with EPIF medium was found ($P < 0.002$).
The mutation rate after EPIF treatment was further analyzed with fluctuation tests to determine when the mutational events took place. Five fluctuation tests were performed with EPIF, produced in serum free medium. As in the mass cultures an enhancement of the mutation rate after EPIF treatment was found. For each mutational event it was determined how many cell generations occurred between treatment and the mutational event. The distribution of the occurrence of the mutational events is different for EPIF and control cultures. In the EPIF treated group more mutational events are found from the first to the fifth generation after treatment compared to the control group ($P < 0.02$). The mutation rate for the generations one to five after treatment was 10-fold higher in the EPIF treated group.
The same experiments revealed that EPIF medium also induced cell growth during the 24 h treatment period when the cells are at a high density. A significant linear correlation between mutation rate and cell growth response was found for EPIF treated cultures.
All results suggest that the recruitment of cells for growth by stress-induced growth factors affects normal control mechanisms and leads to an enhanced mutation rate.

Induction of genetic instability in mammalian cells by ionizing radiation.
As there are several indications for long lasting effects of X-irradiation in mammalian cells the effects of X-irradiation on genetic stability was tested in our GRSL assay system. Ten fluctuation tests altogether with over 8000 parallel cultures have been performed. All radiations were with 6 Gy. The experiments proved to be very heterogeneous in the induction of genetic instability: about half of the experiments displayed a strong indirectly induced mutational response. This response can be expressed as an absolute increase over the spontaneous background or as a relative increase over the background. The relative increase over the background correlated with the cell density of the cultures which were used for the irradiation ($+0.63$ $P=0.07$). This indicates that cells in G_0 might be more sensitive for the induction of genetic instability.

Publications.

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Progress Report

Contract: FI3P-CT920031 Sector: B13

Title: Studies on radiation-induced chromosome aberrations in mammalian cells. 2) Applied aspects.

1)	Olivieri	Univ. Roma "La Sapienza"
2)	Cortés-Benavides	Univ. Sevilla
4)	Palitti	Univ. Viterbo
5)	Savage	MRC

I. Summary of Project Global Objectives and Achievements

A) Low-dose effects and validation of the adaptive response. The evaluation of the genetic risk by low doses of radiation is a very important aspect of radiobiology. In fact, radiobiological dose effects at higher dose and dose-rate levels are satisfactorily elucidated; the relationship of radiobiological effects to very low radiation doses and dose rates, however, still remain ambiguous. Moreover there is now a considerable body of evidence showing that exposure of cells to very low doses of ionizing radiation can, "adapt" them such that they show a reduced response to a subsequent higher dose. However there are apparent anomalies and donor variabilities in published data. A possible validation of the adaptive repair phenomenon accompanying low-dose radiation exposure could have significant impact on the interpretation of low-dose radiobiological effects and in the considerations of attendant risk assessments.

B) Validation of the G_2 phase chromosomal radiosensitive assay in order to identify radiosensitive and cancer prone individuals. It is known that inter-individual variability in human subjects exists for response to the induction of chromosomal aberrations by ionizing radiations. This individual radiosensitivity is becoming more interesting for radiation protection and radiotherapy. Some of these traits are inherited as autosomal recessive disorders which often show cancer proneness and are in many cases results of mutation in genes involved in DNA repair. The G_2 phase of the cell cycle is the most sensitive stage for induction of chromosomal aberrations by ionizing radiation and it has been reported that the yields of x-ray induced aberrations are higher in G_2 lymphocytes and fibroblasts of the cancer-prone syndromes ataxia-telagiectesia (A-T) and Fanconi's anaemia. Differential G_2 radiosensitivity may then be used to identify radiosensitive and radio-resistant individuals. These findings may have relevance for radiotherapy as well as identification of cancer prone individuals.

C) Achievement of a meaningful score of chromatid-type aberrations that can be used for radiation-induced cytogenetic damage. Scores of chromatid-type aberrations are widely used in studies connected with the effects of and protection against ionizing radiation (and many other environmental mutagens).

Unlike chromosome-type aberrations, there is no known cell system where the target-cell population is homogeneous for the production of chromatid-type aberrations. Consequently, the recovered yield of any particular category fluctuates with the time of sampling after treatment. Moreover, the target heterogeneity means that any observed yield will have been profoundly influenced by the population cell kinetics, in particular, mitotic delay. Thus there is no simple relationship between observed yields and the underlying sensitivity patterns within cycle phases. This fact clearly has a bearing upon the interpretation of results from experiments conducted in A) and B) above.

The achievement of new results in these fields are of considerable interest for public health and safety particularly in view of the finding of recent surveys which appear to be revealing carcinogenetic effects, both direct and transmitted, arising from extremely low radiation exposures.

Head of project 1: Prof. Olivieri

II. Objectives for the reporting period

To study the circumstances in which it is possible to obtain different types of interaction (i.e. synergistic response or adaptive response) between low dose irradiation and subsequent mutagenic treatment in G_2 phase human lymphocytes.

For the next reporting period our objectives will be to investigate further the conditions that can effect the types of interaction between the adapting and the challenging dose.

III. Progress achieved including publications

Ten experiments were carried out using cultures of blood from donors which, in previous experiments had displayed an adaptive response or not, and from new donors. Whole blood (0.5 ml) was added to 4.5 ml of RPMI 1640 medium without fetal calf serum, 2 mM glutamine, 100 units/ml penicillin, 100 μg/ml streptomycin, and 2% phytohemagglutinin M (Welcome). The experiments consisted first of exposing cultured human lymphocytes to adapting treatment and subsequently challenging the cells with high doses of X-rays.

In all the experiments the conditioning pretreatment consisted of 0.02 Gy of X-rays. The cells were sebsequently challenged with 0.30 Gy of X-rays and fixed 2 h later. The challenge treatment was given 48-96 h. after stimulation with PHA. 90 minutes before fixation 0.1 ml of colcemid (final concentration 2×10^{-7} M) was added to each culture, and fixation was performed according to standard cytological procedures; for each point examined two parallel cultures were set up. The cells were scored to see wether the prior exposure reduced the number of chromatid and isochromatid breaks induced by the challenging doses.

We considered three main variables: i) the donor ii) the time between the conditioning and the challenge treatment iii) the fixation time after PHA stimulation. The time between the challenge treatment and fixation was the same in all the experiments (2h). The results show that both the donor and the fixation time can modulate the interaction between the adapting and the challenging dose.

All together the data indicate that our donors can be divided into two groups: those who exhibit a synergistic response at fixation time of 50-56h and an adaptive response at 74-

80h, and those who show a reverse pattern, namely adaptive response at 50-60h and synergistic response at 74-80h.

These findings do not agree with those reported by many authors. One possibility is that the mechanism underlying the interaction of low dose irradiation with subsequent mutagenic treatment in G_2 phase human lymphocytes are different from those acting in other phases of the cell cycle, in other materials or for other end points. Moreover, it is conceivable that the possible repair systems induced by the conditioning dose react differently according to the time after PHA stimulation or to the culture conditions such as pH (Olivieri et al. 1992) presence of hydrocortisone or interlenkin-2 (Olivieri and Bosi 1990). Finally the conditioning treatment could act on lymphocyte subpopulations with different progression in the two groups of donors.

Thus the analysis of the yield of chromatid aberrations induced by a conditioning low dose of x ray followed by a challenging dose delivered two h. before fixation, revealed the presence of both synergistic and adaptive interactions between the two treatments.

Publications

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Head of project 2: Prof. Cortés-Benavides

II. Objectives for the reporting period.

The results published up to date on the possible induction of the adaptive response to radiation damage in human lymphocytes conditioned with low doses of ionizing radiation before stimulation (G0) are controversial (Sanderson and Morley, 1986; Shadley et al., 1987; Cai and Liu, 1990; Wang et al., 1991).

A difficulty inherent in the protocol followed by these authors is the possibility that the expression of putative repair genes might interact with the general phenomenon of stimulation induced by a treatment with the lectin phytohemagglutinin (PHA).

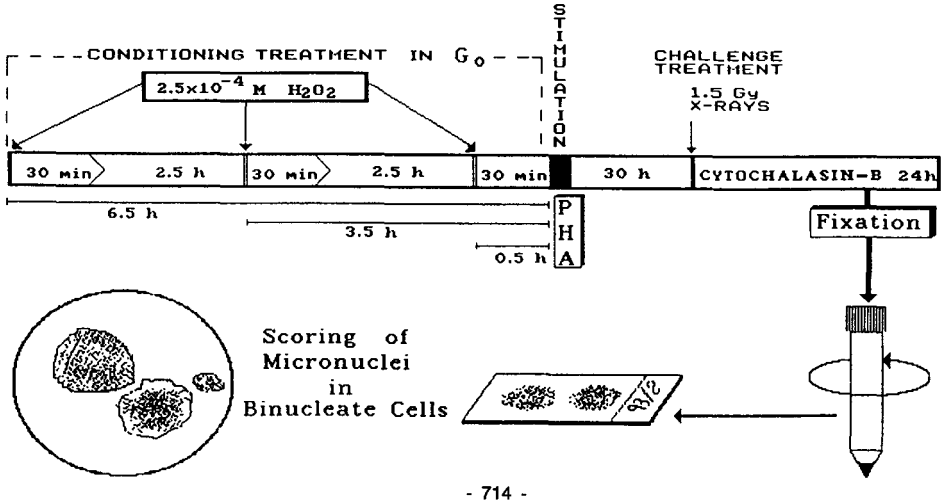
Our main objective for the reporting period has been to analyze the adaptive response by the micronucleus technique (Fenech and Morley, 1985) as reported recently in our laboratory (Domínguez et al., 1993) which offer clear advantages as compared with the classical scoring of chromosomal aberrations.

Our purpose was to treat the cells with H_2O_2 at different times before stimulation and irradiate with X-rays 30 h after stimulation to see whether an adaptive response can be induced.

For the next reporting period our objectives will be to investigate the same question of the possible induction of adaptive response when the conditioning treatment consist in an irradiation with low doses of X-rays at different times before stimulation and compare our results with those reported previously (Sanderson and Morley, 1986; Shadley et al., 1987; Cai and Liu, 1990).

III. Progress achieved.

Fig. 1 illustrates the experimental procedure followed to study the adaptive response in human lymphocytes conditioned with a 30 min pulse with H_2O_2 given at three different times before stimulation and irradiated with X-rays later on.



Human lymphocytes from two healthy donors were conditioned with a dose of $2.5x10^{-4}$ M H_2O_2 for 30 min, ended at 6, 3, or 0 h before stimulation with 2% PHA. We have previously reported that this dose of H_2O_2 is suitable for the induction of the adptive response in stimulated human lymphocytes conditioned with H_2O_2 24 h after stimulation and challenged with X-rays 24 h later (Cortés et al,. 1990; Domínguez et al., 1993).

In this case, the challenge treatment with 1.5 Gy of X-rays was given 30 h after stimulation, and Cytochalasin B was added immediately after irradiation, in order to block cytokinesis and analyze micronucleus formation in cells dividing after X-rays exposure (Fenech and Morley, 1985). The recovery period in Cytochalasin B was for 24 h, and fixation was performed by the standard cytological procedure (Domínguez et al., 1993) after a mild hypotonic treatment to better preserve cytoplasm and allow the scoring of micronuclei. After Giemsa-staining, a total of two thousand binucleated cells were scored, when possible, by two different observers. To determine if the number of micronuclei observed in H_2O_2-conditioned cells was significantly lower than expected, a one-tail t-test was used.

The frequencies of micronuclei in binucleate cells observed in lymphocytes irradiated with 1.5 Gy of X-rays was compared with that found in cells that were previously treated with H_2O_2 in G0 (combined treatment H_2O_2 -irradiation).

Table I shows the results for the two donors. As can be seen, the difference between the expected and observed frequencies of micronuclei was, in general, statistically significant, i.e., there was an apparent adaptive response to radiation damage in human lymphocytes previously exposed to H_2O_2 while in G0. There results are in good agreement with that reported previously by us in lymphocytes conditioned with H_2O_2 24 h after stimulation, following a similar protocol of cytokinesis block (Domínguez et al., 1993).

Concerning the possible induction of the adaptive response by a conditioning treatment in G0 lymphocytes, the results reported to date are controversial. While some authors have not observed a protective effect of pre-exposure to low doses of X-rays against cytogenetic damage (Shadley et al., 1987; Wang et al., 1991) others have reported evidence of an adaptive response (Sanderson and Morley, 1986; Cai and Liu, 1990).

We have found that conditioning G0 human lymphocytes with H_2O_2 seems to protect them from radiation in the frequency of micronuclei in binucleate cells (Table I).

X-ray damage in partly attributed to the production of free radical which are also produced by H_2O_2 (Sobels, 1956). Boreham and Mitchell (1991), on the other hand, have reported that DNA single-strand breaks (ssb), as those efficiently induced by hydroxyl radicals, may be important lesions that signals the induction of the recombinational repair system, that is belived to be the major mechanism that confers resistance to ionizing radiation in yeast.

An explanation for the contradictory results reported by different authors about the induction of the adaptive response in G0 (see above) is not at hand. In our opinion, the hypothesis of a possible interference of the general process of stimulation with the mechanism of adaptation can be ruled out, since adaptation can be observed regardless of the coincidence with stimulation.

The use of the cytokinesis-block method in the present investigation to score micronuclei in binucleate cells, as compared with the scoring of chromosomal aberrations at metaphase, affers the adventage of being able to collet the cells exposed to X-rays at different stages of the cell cycle and allow them to undergo any delay needed to repair before entering mitosis.

Table 1.
Adaptive response in G_0 lymphocytes conditioned with $2.5x10^{-4}$ M H_2O_2 at different times before stimulation.

Donor	Conditioning pretreatment (H_2O_2)	Challenge treatment X-rays	Number of micronuclei observed N°	observed ‰	expected[a] ‰	N° of cells scored
A	None	None	21	10.5	-	2000
	6.5h	None	17	8.5	-	2000
	3.5h	None	20	10.0	-	2000
	0.5h	None	35	17.5	-	2000
	None	1.5 Gy	293	146.5	-	2000
	6.5h	1.5 Gy	218	126.5	144.5[c]	1723
	3.5h	1.5 Gy	152	85.7	146[b]	1772
	0.5h	1.5 Gy	140	70.0	153.5[b]	2000
B	None	None	27	13.5	-	2000
	6.5h	None	17	8.5	-	2000
	3.5h	None	19	9.5	-	2000
	0.5h	None	8	4.0	-	2000
	None	1.5 Gy	336	168.0	-	2000
	6.5h	1.5 Gy	172	114.6	163[b]	1501
	3.5h	1.5 Gy	73	176.0	164[ns]	413
	0.5h	1.5 Gy	310	150.0	174[c]	1777

a. Sum of the two individual treatments minus the control.
b. Observed frequency significantly lower than expected ($P<0.01$) (one-tailed t-test)
c. Observed frequency significantly lower than expected ($P<0.05$).
ns. Observed frequency not significantly lower than expected.

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Head of the project 4: Dr. Palitti

II. Objectives for the reporting period

1. Relationship between the repair processes that take place during the G2 phase of the cell cycle and the chromosomal damage induced by x-ray in A-T cells.

2. To extend the data on the effect of high-LET radiation (neutrons) of G2 post-treatments with hydroxyurea on chromosomal aberrations to compare with the effect of the post-tratments with cytosine arabinoside and aphidicolin already reported.

III. Progress achieved including publications

Lymphoblasts derived from normal (NL),A-T heterozygotes (ATHz) and three A-T patients were x-irradiated (30 and 45 cGy for NL and ATHz,15 and 30 for A-T) and post-incubated with inhibitors of DNA polymerases α , δ (aphidicolin,APC,cytosine arabinoside,ara-C,butylphenyl-guanine,BuPdg) or of dNTPS pool synthesis (hydroxyurea, HU).
The results,expressed as the ratio x-ray induced chromosomal aberrations + inhibitor / x-ray induced chromosomal aberrations,shown in fig.1 shows that a strong increase in x-ray induced chromosomal aberrations was observed in normal and HzAT cells post-incubated with APC,ara-C and HU,but not in the presence of BuPdg,indicating a minor involvement of polymerase α in the repair of x-ray induced damage. No enhancing effect was observed in cells derived from the three A-T patients(fig.2),except for HU post-incubation treatment. These results suggest that the enzymes which can be inhibited by these agents are not directly involved in the repair of the damage induced by ionizing radiations in G2 cells derived from A-T patients,indicating that probably the A-T cells we used lack the capability to trasform the ionizing radiation induced primary DNA lesions into reparaible products or that A-T cells might contain a mutated form of DNA polymerase resistant to the inhibitors.

Publications

A.Antoccia,F.Palitti,T.Raggi,C.Catena and C.Tanzarella. The yield of the fission neutron-induced chromatid aberrations in G2-stage of human lymphocytes:effect of caffeine,hydroxyurea and cytosine arabinoside post-treatments. International J. of Radiation Biology 62:563-570 (1992)

A.Antoccia,F.Palitti,T.Raggi,C.Catena and C.Tanzarella.

Study of the chromosomal damage induced by x-rays in Ataxia Telangectasia lymphoblastoid cells:effect of inhibitors of DNA synthesis and repair. Physica Medica in press.

A.Antoccia,F.Palitti,T.Raggi,C.Catena and C.Tanzarella. Chromosomal damage induced in the G2 stage of Ataxia Telangectasia lymphoblasts by x-ray and fission neutrons: lack of effect of post-irradiation tratment with inhibitors of DNA synthesis/repair. 25th Annual Meeting of the European Society for Radiation Biology,June 10-14,1993,Stockolm,Sweden

FIG 1

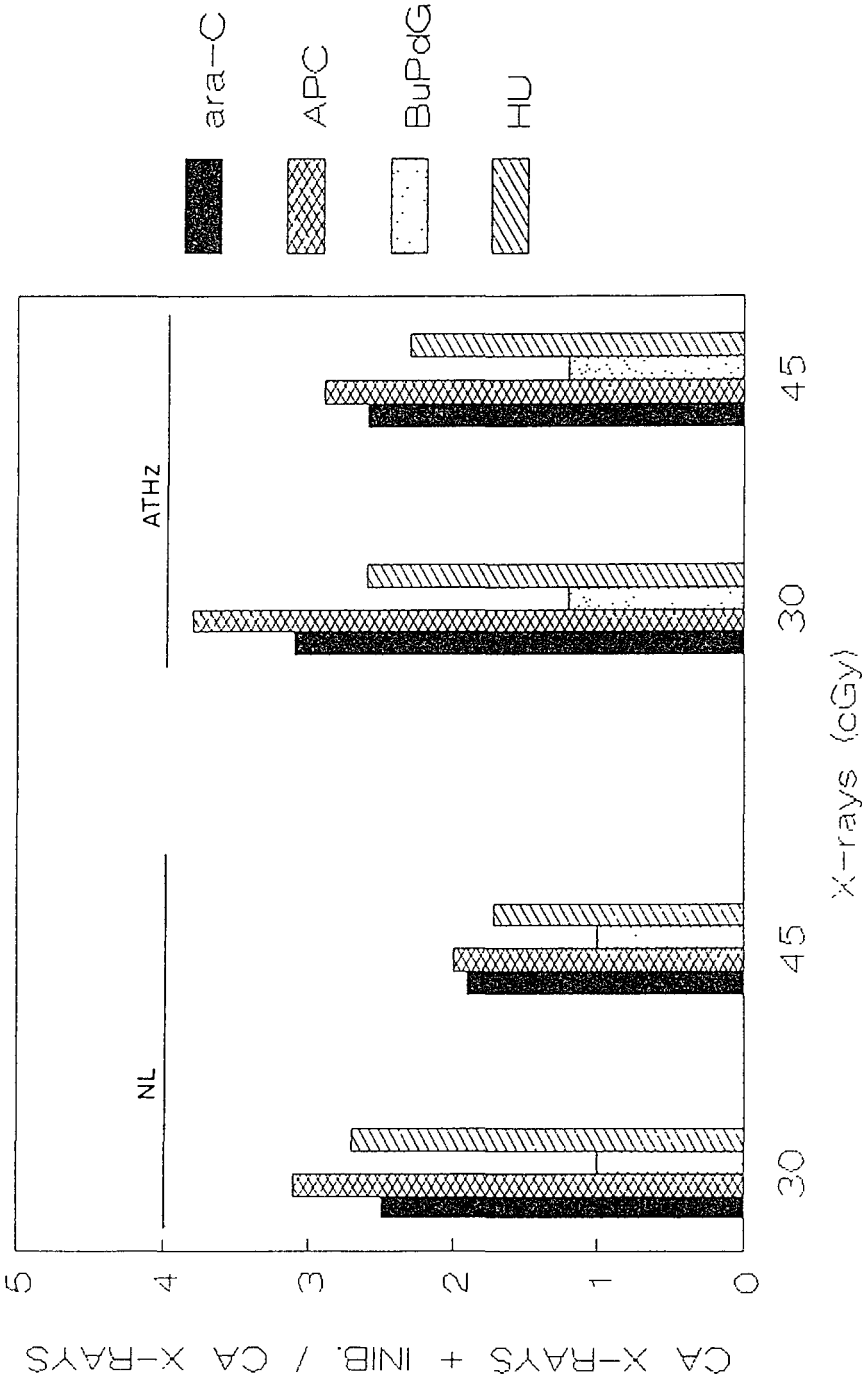
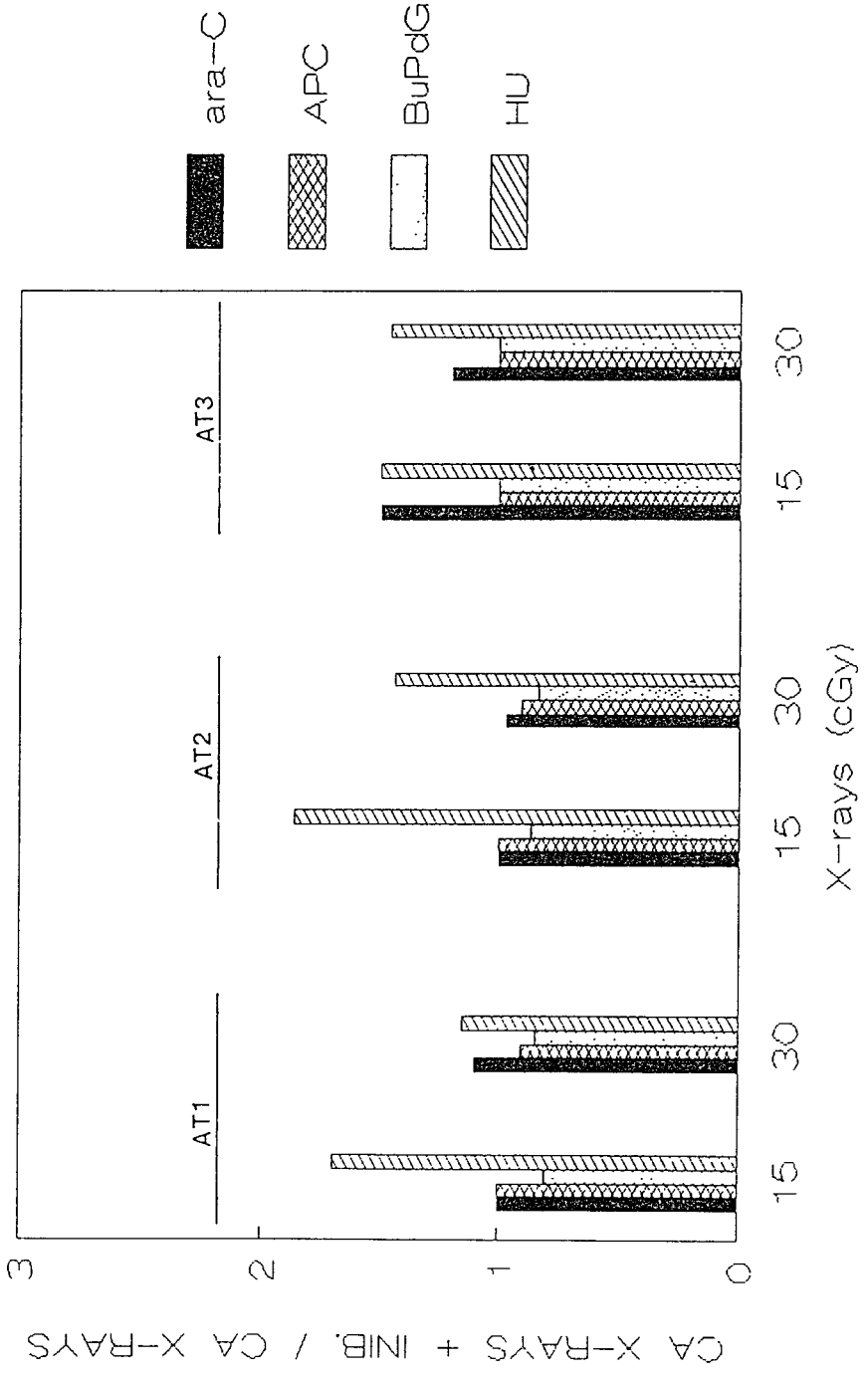

ara-C
APC
BuPdG
HU
NL
ATHz
30
45
30
45
X-rays (cGy)
CA X-RAYS + INIB. / CA X-RAYS
0
1
2
3
4
5

FIG 2


ara-C
APC
BuPdG
HU
AT1
AT2
AT3
15
30
15
30
15
30
X-rays (cGy)
0
1
2
3
CA X-RAYS + INIB. / CA X-RAYS

Head of project 5: Dr J R K Savage.

II. Objectives for the reporting period:

To investigate, in detail, using BrdU techniques, the types and frequencies of X-ray induced chromatid-type aberrations induced by X-irradiation during G_1- and S-phases of the V79-379a Chinese hamster cell line.

To assess the mitotic delay, and study the qualitative and quantitative effects of its mitigation by caffeine.

III. Progress achieved including publications:

Established lines of Chinese hamster cells have been, and are very widely used for the study of radiation-induced aberrations and mitotic delay. Consequently there is an enormous back-up literature available on usage and effects.

We have chosen to use line V79-379A because of its good karyotypic stability (modal 21 chromosomes) and its rapid cycle time (modal cycle transit time ~9h). A bulk stock has been grown and stored in liquid nitrogen. Fresh ampoules are used for each experiment - there is no long term culture maintenance.

A necessary pre-requisite to accurate aberration quantification is accurate classification. Really detailed and critical analysis of chromatid-type aberrations of the kind readily available for plant cells is relatively rare for mammalian cells. Accordingly, our initial work has concentrated on the types and frequencies of aberrations produced during transit of G_1 and late S.

To aid identification of intrachange forms, and to add further dimensions to the subsequent analysis, chromosomes with potential sister-chromatid differentiation (BrdU-substituted, *TB/BB*) were irradiated. This was

achieved by growing the cells for ~2 cycles (17h) in medium containing 10μg/ml BrdU prior to irradiation with various doses of X-rays. Immediately after irradiation, the medium is replaced by identical, pre-warmed medium containing 10μg/ml thymidine instead of BrdU. Although every attempt is made to avoid cell perturbation during manipulation and medium change, fluctuations in control mitotic index indicate that some is always present during the first few hours.

Cultures are sampled at regular 1.0 or 1.5h intervals (each preceeded by colcemid) to cover nearly all of G_1 and mid to late S, and conventional air-dried metaphase spreads are made. These are stained by a modified FPG "harlequin" technique to obtain a 3-way staining differential; *BB* pale, *TB* dark, *TT* very dark. Any cells in S-phase at the time of irradiation (≡ medium change) will take up thymidine and the chromosomes are therefore marked by very dark *TT* patches. Thus, S cells can be distinguished from the "pure" *TB/BB* G_1 cells when scoring aberrations, and, in the multi-sample time experiments, a Fraction of Differentially-stained Metaphases curve (FDM, ≡ FLM) can be constructed for kinetic studies [HARVEY AND SAVAGE, 1992].

Currently, we distinguish G_1 from S cells, but we hope eventually to use the *TT* replication-band patterns to develop a sub-phasing scheme for Chinese hamster S-phase, as we have done for Syrian hamster and human cells under previous CEC contracts. Cells with "partial" harlequin staining (less than two cycles in BrdU), cells with inordinately long G_1 (still *TB/TB*), and any 3rd division cells, are not included for scoring aberrations.

In the first experiment a single dose of 1.5Gy 250kV X-rays was used and 1.5h sampling periods up to 7.5h. Using coded, randomised slides, all categories of aberrations were scored, with particular reference to the intra-arm intrachange types. Analysis of relative frequency and a "Classic" *versus* Revell's "Exchange theory" discussion has been published [SAVAGE AND HARVEY, 1991]. This experiment has been followed up, using a range of doses up to 2.0Gy.

In addition to absolute frequencies per cell and the construction of yield-time curves, achromatic lesions ("gaps"), "breaks", and the presumptive break-points of chromatid interchange were all assigned to light (*l, BB*) or dark (*d, TB*) chromatids.

Many of the "breaks" are probably incomplete intrachanges; some can be seen to be so because they are accompanied by a colour-jump (≡ SCE, but not, of course, an S-dependent SCE in the accepted usage of that term) at the point of discontinuity. In order to obtain the colour-jump break frequency more accurately, extra breaks were scored at each point to bring totals near to 200.

The principal findings from these experiments are [SAVAGE AND HARVEY, 1993]:
1]. The proportion of colour-jump breaks (c*, ~12-14%) is constant, irrespective of dose up to 2.0Gy (and from more recent work, of BrdU concentration from 1-10μ/ml). Even more surprising, a sample of "spontaneous" breaks from unirradiated controls had exactly the same frequency.
2]. No evidence that either achromatic lesions, breaks, or type 2 intrachanges are more frequent in *l* (*BB*) chromatids as might be expected from bromouracil radiosensitization ($l/d \approx 1.0$).
3]. In contrast, breaks involved in *inter*changes predominate in *l* chromatids ($l/d \approx 2.0$) suggesting differential sensitivity.
4]. About 12% of interchanges involve a colour-jump break as one of the participants.

The constancy of the c* break frequency implies that *intra*changes form a *fixed* proportion of all breaks, irrespective of absolute number. One would

have anticipated from Classic "breakage-and-reunion" theory that the relative proportions of "one-hit" and "two-hit" events would change with dose. The inference is, therefore, that simple, unrestituted breaks are making a negligible contribution to observed breaks.

Because of our interest in the effects of delay, we repeated the experiments at 1.5Gy, using sampling times at hourly intervals up to 7h, with and without 400μg/ml (2.1×10^{-3} M) caffeine. Such a dose cancels, or mitigates mitotic delay in this material. At the same time we expected an increase in breaks, presumably because the cancelled delay reduces time for repair. If these extra breaks result from failed restitution, a dramatic drop in relative c* frequency should be observed.

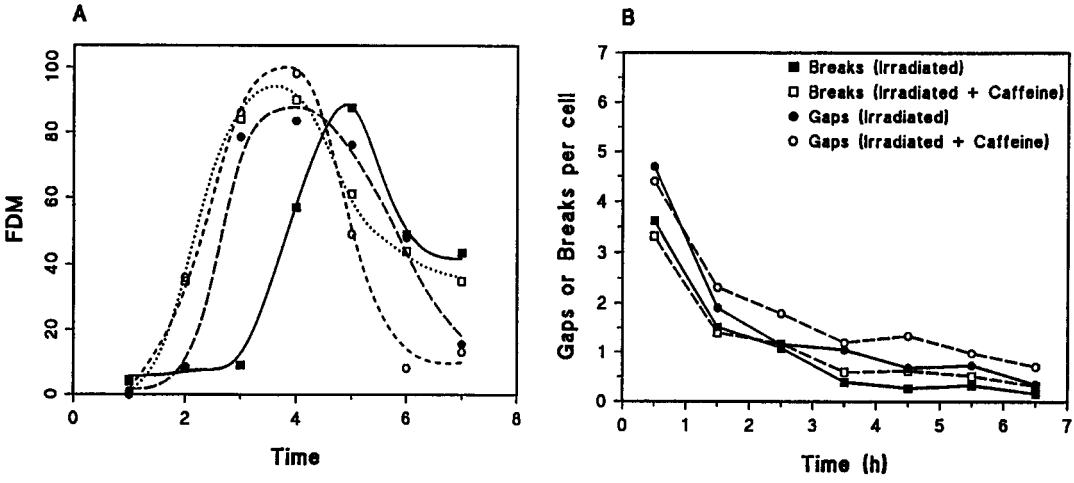


Figure 1. **A:** FDMs. □ Control; ○ Control+caffeine; ■ irradiated; ● irradiated+caffeine.
B: Yield-time curves for gaps and breaks.

Figure 1A shows the observed FDMs with and without caffeine, showing that delay is largely cancelled by the drug. Figure 1B shows that the frequencies of gaps and breaks are almost unaffected, marginal significance of differences are only obtained if the whole yield-time curves are tested. This effectively divorces enhanced breakage from mitigated delay in this experiment. The presence of caffiene did not change the c* proportion, nor the *1/d* ratio for gaps and breaks, but the *1/d* ratio for interchanges was slightly lower (~1.6) for both caffeine and non-caffeine, compared with previous experiments [HARVEY AND SAVAGE, 1993].

The fact that delay cancellation has had very little effect on the yield time curves is encouraging from the quantitative point of view. From initial theoretical work [SAVAGE AND PAPWORTH, 1991] we might have expected otherwise.

We have now begun a similar investigation using ^{238}Pu α-particles. Here, because of the highly track-structured and high-LET nature of this radiation, mixtures of irradiated and unirradiated cells will exist at all usable doses, and will produce, amongst other things, differential cell delay, changing cell mixtures continuously with time of sampling. At the same time, we will be able

to make a detailed comparison of types and frequencies of aberrations with those we have found for X-rays.

Publications:

HARVEY AN, SAVAGE JRK (1992): SIMPLE CELL CYCLE ANALYSIS IN SECOND DIVISION CELLS. CLIN.CYTOGENET.BULL. 2: 94-96.

HARVEY AN, SAVAGE JRK (1993): A CASE OF CAFFEINE-MEDIATED CANCELLATION OF MITOTIC DELAY WITHOUT ENHANCED BREAKAGE IN V79 CELLS. MUTATION RES. (IN PRESS).

SAVAGE JRK, HARVEY AN (1991): REVELL REVISITED. MUTATION RES. 250: 307-317.

SAVAGE JRK, HARVEY AN (1993): INVESTIGATION OF ABERRATION ORIGINS USING BRDU. IN OBE G, NATARAJAN AT (EDS): "CHROMOSOMAL ABERRATIONS: ORIGIN AND SIGNIFICANCE." (IN PRESS).

SAVAGE JRK, PAPWORTH DG (1991): EXCOGITATIONS ABOUT THE QUANTIFICATION OF STRUCTURAL CHROMOSOMAL ABERRATIONS. IN OBE G (ED): ADVANCES IN MUTAGENESIS RESEARCH.3. SPRINGER-VERLAG, PP162-189.

IV. Objectives for the next reporting period:

A detailed comparative study of X-ray and ^{238}Pu α-particles for the production of chromatid-type aberrations in this V79-379A line of hamster cells.

To evaluate the effects of the differential mitotic delay which arises from the presence of a mixture of irradiated and unirradiated cells within each cycle phase consequent upon use of a highly track-structured high-LET radiation.

To use DSB-rejoin inhibitors and mutants to investigate further the constancy of the relative c* frequency.

Progress Report

Contract: FI3P-CT920042 Sector: B13

Title: Carcinogenic effects of low radiation doses and underlying mechanisms.

1)	Davelaar	Univ. Leiden
2)	Coppola	ENEA
3)	Bentvelzen	TNO - Delft
4)	Masse	CEA - FAR
5)	Chmelevsky	GSF
6)	Zurcher	TNO

I. Summary of Project Global Objectives and Achievements

In this joint project the emphasis is placed on the carcinogenic effects of low radiation doses and the underlying mechanisms. The influence of various exposure or host conditions on the probability of cancer development have been studied. The project involves the analysis of data from recently accomplished experiments, the completion of on-going experimental studies, and the performance of a limited selections of new series. Mechanistic studies of radiation induced neoplasia using animal models are presently confined to thymic lymphoma, myeloid leukaemia, osteosarcoma and mammary carcinomas in rodents. New insights into carcinogenesis emerging from molecular biological investigations, may provide, by identification of a variety of steps and pathways, much needed information on the sensitivity to radiation among different cell types, and on cell cycle dependence.

The progress achieved by the various participants can be summarized as follows:

1. AZL: The computer programs LifePrep and LifeStat have been developed to serve as a standard tool for the analysis of dose-effect relationships in animal tumour induction data. The program has been applied to data on WAG-Rij rats from the ITRI-TNO institute in order to study mammary carcinogenesis among others in view of radiation protection questions in general or the risk for tumour induction in large scale breast screening programs in particular. The logistics of tumour incidence studies has been correlated with the activities concerning the European Radiobiological Archive of Animal Experiments.

2. ENEA: Results of in-vivo studies of carcinogenesis in long-living mice after single or fractionated doses of fission neutrons have been analyzed. For the induction of lung tumours no significant overall dose-fractionation effect has been observed. However, for the lung, as for solid tumours in other organs the incidence at a dose of 70 cGy appears conmsistently lower for fractionated neutron doses than for acute exposures.

3. ITRI-TNO: Radiation carcinogenesis has been studied in the mammary gland of WAG-Rij rats with single doses at different ages and fractionated exposures with

small fraction sizes. For the hormone treated animals fraction sizes of 2.5 and 10 mGy do not produce a significantly increased risk at a cumulated dose of 1 Gy. The molecular biological aspect was investigated by isolation of DNA from 10 rat mammary tumours. Two tumours had a mutation in codon 12 of the K-ras oncogene. The F1 hybrid of the susceptible SD and resistant BN strain proved to be fully susceptible to the induction of mammary tumours by X-rays. DNA has been collected from the animals and will be used for genetic typing.

4. CEA: Experiments have been performed on lung tumour induction in rats after exposures to radon at 25 WLM delivered at dose rates of 2, 100 and 150 WL. Experiments on tumour induction after 2.5 cGy neutrons from 252-Cf at dose-rates 950 and 3.6 μGy per hour are still in progress. The steps occurring during lung carcinogenesis after radon inhalation have been characterized by measuring expression of cytochrome P450 1A1 with biochemical methods and by visualizing it by immunohistological methods.

5. GSF: The analysis methods for tumour induction in animals are performed in subsequent steps, notably the Kaplan-Meier estimators, the Cox regression for a joint analysis and the use of analytical functions. This effort can be considered as complementary to the valuable work performed in Brussels to create a databank of results from animal experiments in various European laboratories.

6. IVVO-TNO: Gross necropsy material of about 200 rats has been collected for microscopic examination. From a comparison of pathology criteria propagated by the American Society of Toxicologic Pathologists (STP) or the Fraunhofer Institute (Reni/WHO) it appears that the first classification is more generally acceptable. A start has been made to introduce the STP terminology into the IVVO-TNO Path-Data files.

Head of project 1: Dr. Davelaar

II. Objectives for the reporting period

The program **LifePrep** has been developed under a previous contract to provide a conversion of tumour incidence data into an appropriate format. The generation of the computer program **LifeStat** for the analysis of animal carcinogenesis data has been performed at the Department of Clinical Oncology in cooperation with GSF. Extensive documentation for the use of this program is produced and an 80-page description of the underlying principles of the data analysis can be provided upon request. The program currently encompasses the analysis of single dose and fractionated experiments and can be extended to the analysis of protracted experiments as performed at the CEA-FAR. The program is produced in such a way that it can be distributed among the various European institutes, which cooperate within this contract. In collaboration with ITRI-TNO the error propagation for tumour induction data of many small fractions is under investigation.

III. Progress achieved including publications

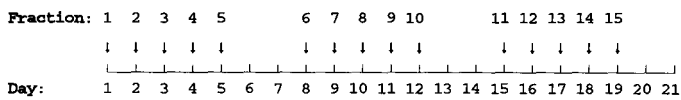
The computer programs **LifePrep** and **LifeStat** have been developed to serve as a standard tool for the analysis of dose-effect relationships in animal tumour induction data. The program has been applied to data on WAG-Rij rats from the ITRI-TNO institute in order to study mammary carcinogenesis a.o. in view of radiation protection questions in general or the risk for tumour induction in large scale breast screening programs in particular. A more general application to carcinogenesis data from other European institutes is envisaged.

One outstanding question with respect to the analysis of dose-effect relationships has been the equivalence of the parametric model with Weibull functions, as used with ITRI-TNO data, and the maximum likelihood analysis or the non-parametric proportional hazard model. **LifeStat** has been used to show the validity of both models, as applied to the mammary carcinogenesis data

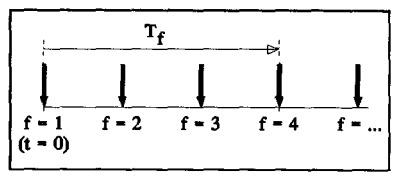
from ITRI-TNO (Davelaar et al, 1991).

For the CEN-FAR data, which has been studied in cooperation with GSF, we found some important differences with respect to pathology as compared to the ITRI-TNO data. In an attempt to facilitate the agreement on a standard pathological classification scheme the conversion program **LifePrep** (Weeda et al, 1992) needed to be developed. It provides versatile selection and grouping procedures of animal data with different pathology as well as a conversion of the data to the format of the **LifeStat** program. In the reporting period the pathology classification and data grouping have also been discussed for the *European Radiobiological Archive of Animal Experiments* (Gerber and Gössner, 1993).

For the TNO data with many small fractions (Bartstra et al 1993) **LifeStat** is used in a theoretical study. Fractionated experiments may be fitted with multiple Weibull functions. For fractionated analysis the irradiation scheme has to be specified as illustrated in the scheme below. The total dose of a certain experiment is f.i. delivered in 15 fractions: one fraction at each day of the week except on saterday and sunday. Time schedule:



A multiple Weibull function is expressed as a summation of fractional Weibull functions with equal weight and an additional forward shift in time. The supplementary time-shift parameter is the fraction delivery time T_f. The first fraction, T_1, is always delivered at $t = 0$, the second fraction at $t = T_{unit}$, and so on as is illustrated by the following figure.



The additional time-shift parameter is added to the expression of the cumulative survival *S(t)*:

$$S(t) = \frac{1}{F}\sum_{f=1}^{F} e^{-\left(\frac{t-T_f-\gamma}{\alpha}\right)^{\beta}}, \text{ or } \quad H(t) = \frac{1}{F}\sum_{f=1}^{F}\left(\frac{t-T_f-\gamma}{\alpha}\right)^{\beta}$$

f - fraction sequence number (f = 1..F),

F - the number of fractions,

T_f - time of fraction delivery.

The equation for estimation of the maximal log-likelihood value results in:

$$\sum_{g=1}^{N_g}\left[\frac{N_{fg}}{\beta} + \sum_{i=1}^{N_{fg}}\frac{1}{F}\sum_{f=1}^{F}\ln(t_{ig}-T_f) - N_{fg}\frac{\sum_{k=1}^{N_{eg}}\frac{1}{F}\sum_{f=1}^{F}\left(\ln(t_{kg}-T_f)\ (t_{kg}-T_f)^{\beta}\right)}{\sum_{k=1}^{N_{eg}}\frac{1}{F}\sum_{f=1}^{F}(t_{kg}-T_f)^{\beta}}\right];$$

Where: F_g - the number of fractions of dataset g.

It may be observed that terms including event times are modified to a weighted summation over all fractions of the event time minus the fraction delivery time. Differences between the single dose and the fractionated analysis could have important consequences for the error propagation and statistical significance of dose-effect relationships, which is subject to further study.

Publications

Davelaar, J., Weeda, J., Broerse, J.J., "Analysis of animal carcinogenesis data by various mathematical methods", Rad.Env.Biophys. 30, 249-252, 1991.

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Davelaar J., Weeda J., Bartstra R.W., Van Bekkum D.W., Broerse J.J.: "Risico's van tumorinductie na blootstelling aan lage doses", NVS News, (1993).

Gerber G.B., Gössner W., "*European Radiobiological Archive of Animal Experiments (ERA)*", Part I, List of Communicated Experiments, (1993).

Head of project 2: Prof. Coppola

II. Objectives for the reporting period

- Carcinogenic effect of fractionated doses of fission neutrons on $BC3F_1$ male mice.
- Myeloid leukaemia in CBA/Cne mice: a) induction by acute and fractionated doses of fission neutrons; b) genetic factors influencing radiosensitivity.
- Ovarian tumour induction with partial-body irradiation.
- Transformation assays of human cells.

III. Progress achieved including publications

The work in progress at ENEA Casaccia Laboratory includes studies of the influence of dose, dose rate and radiation quality on the induction of malignant transformation in different strains of mice of both sexes and various ages, with particular attention to low dose exposures. It concerns first the analysis of the results of recent experimental series, the completion of on-going experiments, and the performance of a limited selection of new series.

In order to investigate the influence of the irradiation temporal regime on the effectiveness of low neutron dose exposures, the results of recent experimental *in vivo* studies of carcinogenesis in long-living $BC3F_1$ male mice after fractionated doses of fission neutrons were analysed. About 1200 $BC3F_1$ male mice, subdivided in 9 groups, had received five equal daily dose fractions of fission neutrons from the RSV-TAPIRO reactor, corresponding to cumulative doses of 2.5 to 70 cGy. Data treatment included the correction for competitive risks and the analysis in terms of cumulative mortality, death-rates for specific causes, and trend. A comparison was made with data for acute exposure of $BC3F_1$ mice at comparable doses of fission spectrum neutrons, for critical endpoints. The results for the lung are shown in Fig. 1. This analysis indicates no significant overall dose-fractionation effect on tumour induction. However, for the lung, as for solid tumours in other organs the incidence at a dose of 70 cGy appears consistently lower for fractionated neutron doses than for acute exposures. In addition, the case of acute myeloid leukaemia remains somewhat ambiguous; however, this disease is very rare in our hybrid mice.

For the above reasons, a new experiment has been initiated mainly to investigate the induction of acute myeloid leukaemia (AML) in CBA/Cne male and female mice after acute and fractionated doses of fission neutrons. The principal aim is to obtain information about the influence of neutron dose fractionation on the induction of this relevant systemic disease related to well identified cells, i.e. haemopoietic cells, in a mouse strain which has proved to have a good response to radiation for this endpoint. The collection of results for the acute irradiation is presently in progress. Irradiation with fractionated doses is planned beginning after summer 1993.

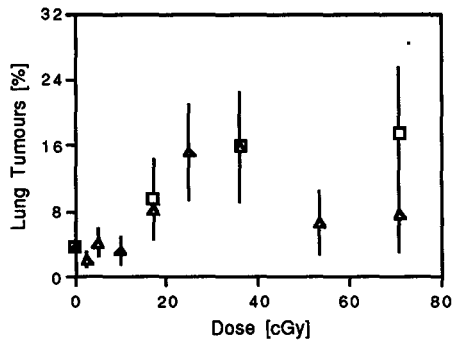

Fig. 1. Percent incidence of lung tumours in $BC3F_1$ male mice whole-body irradiated with fractionated (triangles) or acute (squares) fission spectrum neutrons. Bars are SE.

These experiments will also provide material for studies of the genetic sensitivity to AML in rodents to be carried out in collaboration with other European laboratories. There is in fact, accumulating evidence about the influence of genetic factors on carcinogenesis, which encourages large cooperative efforts towards studies of genetic susceptibility and consequently predisposition to carcinogenesis. As a first step, the restriction length polymorphism of telomeric-like sequences will be determined in the CBA/Cne mouse colony. To this aim, a total of about 50 spleens from young adult male mice will be frozen immediately after excision and sent to Dr. M. Janowski (VITO, Mol) on dry ice for this type of analysis, by the end of September 1993.

In addition to studies of genetic factors influencing the susceptibility to carcinogenesis in inbred strains, it is considered very important to investigate the possibility of regulation by a number of genes of the susceptibility to carcinogenesis in a heterogeneous experimental population, as this may have direct implication for the human case. To this aim, an experimental approach is being followed consisting of a bi-directional selective breeding for susceptibility and resistance to two-stage skin carcinogenesis, initiated by DMBA and promoted with TPA. The character chosen for the assortive mating is the number of papillomas at the end of a promotion period of about 50 days. The selection was started from a highly heterogeneous mouse population, produced by inter-crossing of eight inbred strains of mice. An increasing difference in the frequency of skin tumours was observed between the resistant and the susceptible lines through successive generations. This confirms the polygenic regulation of these characters, though some genes may have a dominating role. Selection experiments will be continued until a maximum interline separation (selection limit) is reached, to obtain mice homozygous for all loci regulating the investigated character. When this is obtained, new experimental work is scheduled to investigate if the same genes controlling the selected characters might have also affected the life-span, the spontaneous incidence and the radiation-

induced tumours (not only skin tumours) in resistant and susceptible lines of mice irradiated either locally or whole-body.

An experimental study is planned on ovarian tumour induction with irradiation of only one ovary, in order to investigate the influence of hormonal imbalance caused by irradiation on the induction of tumours in the unirradiated ovary. It is also intended to study the possible association of characteristic tumours, such as lymphoma and myeloid leukaemia, with paternal irradiation of mice before conception.

In addition to the continuation of the on-going work on murine fibroblastic C3H10T1/2 cells, studies of neoplastic transformation induced by radiation in human derived cell lines from various epithelial tissues are in progress by the same research team within another coordinated project (contract FI3P-CT92-0043) and are described in that progress report.

For the interpretation of radiobiological experiments the knowledge of the microscopic distribution of energy deposition remains of a primary importance. Therefore the results of studies of the complete microdosimetric characterisation of the radiation fields present in the actual irradiation facility and in a new facility have been published.

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6. Saran, A., Pazzaglia, S., Pariset, L., Coppola, M., Di Majo, V., Rebessi, S. and Covelli, V. Dose fractionation dependence of C3H10T1/2 cell transformation by fission spectrum neutrons. 40th Annual Meeting of the Radiation Research Society. Salt Lake City, 1992.
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11. Saran, A., Pazzaglia, S., Pariset, L., Rebessi, S., Coppola, M., Di Majo, V., Covelli, V. Cell-cycle dependence of C3H10T1/2 cell transformation by fission spectrum neutrons. 41th Congress of the Radiation Research Society. Dallas, March 1993.
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Head of project 3: Dr. P. Bentvelzen

II. Objectives for the reporting period

Continuation of research on modifying factors involved in the induction of mammary cancer by radiation in the rat.
To evaluate changes in susceptibility for the induction of mammary carcinomas with increasing age at exposure, with hormone administration (estrogen E_2).
To evaluate possible differences in relative risk for the induction of mammary carcinomas between fractionated irradiation and single dose with hormone administration (E_2).To investigate the possible changes in susceptibility with increasing age during the fractionation period, as described above. At present, a second experiment is analyzed, with the same objectives, but now carried out in rats without hormone administration.

Molecular biology of Radiation-induced mammary cancer in rats
To detect in radiation-induced tumours activating mutations in ras-oncogenes by means of the polymerase chain reaction (PCR) using intron-specific primers followed by dot blot hybridization with selected oligonucleotides.
To improve the technique for the detection of homozygotes deletions (involving subtraction hybridization and sequence-independent PCR).
To detect activated oncogenes in radiation-induced mammary tumours by means of the NIH/3T3 transfection nude mouse oncogenicity assay. To make the technique for the detection of homozygous deletions applicable to fresh tumour samples.

Genetics of susceptibility to radiation induced mammary cancer
To identify a susceptibility gene by means of a crossing programme between a susceptible and a rat strain resistant to radiation-induction of mammary cancer.
To observe the irradiated progeny of various crosses for the appearance of tumours. To type genetically the animals by means of hyperpolymorphic tandem repeat sequences in order to find an association with a putative susceptibility gene for radiation-induction of mammary cancer.

Progress achieved including publications

Continuation of research on modifying factors involved in the induction of mammary cancer by radiation in the rat.
Female WAG/Rij rats, in which the hormone levels were artificially increased (administration of E_2) have been subjected to fractionated exposure with Cs-137 gamma rays, with fraction sizes of 2.5 mGy, 10 mGy and 40 mGy up to cumulated doses of 1 and 2 Gy. In order to investigate possible changes in susceptibility with age, single dose irradiations were performed with doses of 1 and 1 Gy for animals with an age of 8, 36 and 64 weeks. The animals were observed during their total life time, i.e. up to a period of approximately 3 years. The tumour incidence data in course of time have been analyzed with actuarial statistics such as the Kaplan-Meier method and analytical methods such as the Weibull distribution. The relative risks for the induction of carcinomas after fractionated irradiation are summarized in Table 1.

Table 1: Relative risks for the induction of carcinomas in WAG/Rij rats at increased hormone levels after fractionated irradiation with gamma radiation.

total dose	*fraction size*		
	2.5 mGy	*10 mGy*	*40 mGy*
0 Gy	1	1	1
1 Gy	1.4 ± 0.5	1.3 ± 0.5	3.3 ± 1.3
2 Gy	3.3 ± 1.1	2.2 ± 0.8	4.5 ± 1.8

It can be concluded that fraction sizes of 2.5 and 10 mGy do not produce a significantly increased risk at a cumulated dose of 1 Gy. The relative risks for the induction of carcinomas

after single dose irradiation performed at an age of 8, 36 and 64 weeks are presented in Table 2. For both dose levels an appreciable decrease in the relative risk is observed for the irradiation at increased age.

Table 2: Relative risks for the induction of carcinomas in WAG/Rij rats at increased hormone levels after single dose irradiation with gamma radiation.

total dose	*age at exposure*		
	8 weeks	*36 weeks*	*64 weeks*
0 Gy	1	1	1
1 Gy	4.8 ± 1.6	0.62 ± 0.2	0.40 ± 0.13
2 Gy	7.5 ± 2.6	1.0 ± 0.3	0.40 ± 0.13

Molecular biology of radiation-induced mammary cancer in rats
DNA was isolated from ten rat mammary tumours, induced by Cs-137 radiation. By using intron-specific primers the first exon of either K-, H- or N-ras oncogenes was amplified by means of the polymerase chain reaction (PCR). Using selected oligonucleotides the PCR-products were tested by dot blot hybridization for missense mutations in either codon 12, 13 or 61 of the three oncogenes. Two tumours had a mutation in codon 12 of the K-ras oncogene. This result indicates that mutation of a ras-oncogene is not a major pathway of radiation-induction of mammary cancer in the rat.
The technique for the detection of homozygous deletions, involving subtraction hybridization followed by sequence-independent PCR, has so far been applied to cell lines of radiation-induced tumours. This technique yielded six different PCR-products ranging in size from 0.3 to 1.2 kilobase.
With either PCR product as a probe in Southern blot hybridization experiments on normal rat DNA the same pattern was found. These products represent a moderate repeat sequence. This was also concluded form fluorescent *in situ* hybridization experiments on normal rat fibroblasts with the PCR-products as a probe. DNA from radiation-induced tumours produced a Southern blot pattern comparable to that of normal DNA. However, some bands were lacking. It was estimated that a fragment of approximately 4 kilobase in two different radiation-induced tumour lines was lacking.

Genetics of susceptibility to radiation induced mammary cancer
The F_1 hybrid of the susceptible SD and resistant BN strain proved to be fully susceptible to the induction of mammary tumours by X-rays. The F_1 was back crossed to either BN (105 female offspring) or SD (93 female offspring). The animals were given total body irradiation (2 Gy X-rays). So far, 21 per cent of the backcross to SD and 13 per cent of the backcross to BN developed a mammary tumour. It is still to early to draw any conclusion form these results. DNA has been collected from all these animals which will be used for genetic typing.

Broerse JJ, Van Bekkum, DW. Radiation carcinogenesis studies in animals. Advantages, limitations and caveats. In: Advances in Radiation Biology 1992, 16: 199-213.
Broerse JJ, Davelaar J, Weeda J, Bartstra RW and Van Bekkum DW. Mammary carcinogenesis in the rat after low-dose irradiation. In: Low Dose Irradiation and Biological Defense Mechanisms (eds. Sugahara, T., Sagan LA and Aoyama T). Excerpta Medica 1992, pp. 211-214.
Van Klaveren P, De Bruyne JA, Van der Winden J, Kal HB, Broerse JJ, Van Bekkum DW, Bentvelzen P. Molecular-biological detection of genetic alterations in radiation induced rat tumors. Voordracht Int. Seminar "Molecular Mechanisms in Radiation Mutagenesis and Carcinogenesis", Doorwerth, 19-22 april 1993. Proceedings in Press. Editors: K Chadwick en HP Leenhouts (CEC, RIVM, DOE).
Van Klaveren P, De Bruyne J, Van der Winden J, Kal HB and Bentvelzen P. A common deletion in two gamma ray induced rat pulmonary tumor cell lines. Anticancer Res. In press.

Head of project 4: Dr. Masse

II. Objectives for the reporting period for CEA Fontenay aux Roses.

The objectives for 1992-1993 were

* to determine lung tumor incidence after irradiation at low doses of radon and its daugthers with low dose rates.

* to initiate a "banque" of neoplastic and preneoplastic lesions obtained after various carcinogen and cocarcinogen treatments,

* to characterize the steps occuring during histogenesis of tumors at high frequency.

From the obtained results, the main objectives for 1994-1995 will be:

* determination of tumor incidence after exposure to neutron,

* continuation of the "banque" and initiation of genetic studies to characterize mutations in tumor cells according to carcinogenic or cocarcinogenic treatment,

* determination of the role of cytochrome P450 1A1 during cocarcinogenesis produced by inducers of this enzyme administered after exposure to radon and its progeny.

III. Progress achieved including publications

Experiments on lung tumor induction after exposures to radon at 25 WLM delivered at dose rates of 2, 100 and 150 WL have been achieved. Experiments on tumor induction after 2.5 cGy neutron from 252Cf at dose rates of 950 µGy and 3.6 µGy per hour are still in progress.

The various treatments used, irradiation, chemical compounds, metals and fibers have allowed to obtain tumor sample for initiating the "banque" with fresh frozen tissue or formaldehyde fixed tissue embedded in paraffin.

The steps occuring during lung carcinogenesis induced by 5-6 benzoflavone (bNF) following 1000 WLM radon inhalation have been characterized by measuring expression of cytochrome P450 1A1 with biochemical methods and by visualizing it with immunohistological methods.

Similar inductions of CYP 1A1 in lung tissue were induced in radon exposed or unexposed rats. During the bNF treatment (6 intramuscular injections, 25 mg/kg, at 2 week interval), Ethoxy Resorufin O Deethylase (EROD) activies were 30 to 40 times higher than controls. This induction remained at a nearly constant level throughout the treatment and during several months after its end, up to 7 months. Rats only exposed to radon had EROD levels similar to that measured in controls. These enzymatic results were confirmed by visualizing CYP 1A1 on western blots.

In rats treated with bNF, immunohistochemistry have shown CYP 1A1 induction in endothelial cells, non ciliated bronchiolar and bronchial cells and in type II alveolar cells. Over expression was observed in cells located at the bronchiolo-alveolar junction which are the target cells for the cocarcinogenic process. In radon exposed rats, these cells became hyperplastics and reached adjacent alveolar ducts and alveoli still overexpressing CYP 1A1. The CYP expression disappeared when cells transformed into epidermoïd metaplasia. These results suggest that CYP 1A1 expression is a primeval step during the cocar-

cinogenesis.

* Experiments are in progress to characterize the role of bNF metabolites. For that purpose, rats have been treated with two kinds of inducers, methylcholanthrene, which is metabolized under mutagenic compounds and 2-3-7-8 tetrachlorodibenzo paradioxin which is not metabolized.

Publications:

Expression of cytochrome P-450 1A1 in rat lungs during carcinogenesis induced by radon inhalation followed by 5-6 benzoflavone administration. E. Douriez, P. Kermanac'h, P. Fritsch, J.P. Morlier, G. Monchaux and P. Laurent. Oral presentation at the 24th annual meeting of the European Society for Radiation Biology, October 4-8, 1992, Erfurth, Germany.

J.P. Morlier, M. Morin, J. Chameaud, R. Masse, S. Bottard and J. Lafuma. Importance du rôle du débit de dose sur l'apparition des cancers chez le rat après inhalation de radon. C.R. Acad. Sci. Paris, t. 315, série III, 463-466, 1992.

J.P. Morlier, M. Morin, G. Monchaux, P. Fritsch, J.F. Pineau, J. Chameaud, J. Lafuma and R. Masse. Lung cancer incidence after exposure of rats to low doses of radon: influence of dose rate. Oral presentation for the first international workshop on Indoor Radon Remedial Action, paper submitted to Radiation Protection Dosimetry.

Report for project: Analysis of animal experiments
Laboratory: GSF-Neuherberg
Head of project: Dr. D. Chmelevsky (5)

Objectives for the reporting period.

Description of methods of analysis applicable to animal experiments.

Progress achieved.

We have prepared in parallel to the suite of programs elaborated in Leyden a description of methods usable in the analysis of animal experiments. This includes a description of the various steps:

1. The simple Kaplan-Meier estimator for individual groups of animals submitted to the same treatment and having the same characteristics (age, sex, etc. for example).

2. The Cox regression for the joint analysis of several groups of animals or of one group of animals with different characteristics (different treatments).

3. The use of analytical functions for the rates, such as the Weibull function, to be fitted by maximum likelihood to one or several groups of animals.

4. The methods applicable to incidental data as they occur with non lethal and non observable tumours.

This effort as well as the effort in Leyden is to be seen as complementary to the very valuable work done in Brussels to create a date bank of results from animals experiments performed in various European laboratories.

Objectives for the next reporting period.

We are presently applying this work to an experiment performed at the GSF on dogs which were irradiated following bone marrow transplant.

Publication: Manuscript in preparation

Head of project 6: Dr. Zurcher

II. Objectives for the reporting period

* To collect materials from ITRI studies on the low dose irradiation project and from the crossing programme, using rat strains with high or low susceptibility to radiation induced mammary carcinogenesis, for microscopic examination.

* To compare diagnostic pathology criteria propagated by the American Society of Toxicologic Pathologists (STP) or Fraunhofer Institute (Reni/WHO) with those in use in the participating laboratories and to inplement one well defined internationally acceptable diagnostic classification system for future studies.

* To further define and apply criteria for estimating lesions which significantly contribute to the death of the animal.

III. Progress achieved including publications.

* Gross necropsy material of about 200 rats has been collected for microscopic examination in the last contract year.

* From comparison of proposals for diagnostic criteria for rat neoplastic lesions (Reni/WHO an STP, as far as available) it appears that the STP classification is advantageous as it is more in line with our present diagnostic terminology and will most probably more generally be accepted. A start has been made to introduce the STP terminology in our PathData files.

* Criteria have been developed for determining whether a lesion has significantly contributed to the death of the animal. These are mainly based on quantitative, clinical and gross necropsy data and not on the microscopic distinction between benign and malignant tumor growth. These criteria are being applied in ongoing studies to be completed in the following year.

Progress Report

Contract: **F13P-CT920043** **Sector:** **B13**

Title: Measurement of oncogenic transformation of mammalian cells *in vitro* by low doses of ionizing radiation.

1)	Mill	Nuclear Electric
2)	Frankenberg	Univ. Göttingen
3)	Roberts	UKAEA
4)	Tallone Lombardi	Univ. Milano
5)	Kellerer	GSF
6)	Saran	ENEA

I. Summary of Project Global Objectives and Achievements

I.1 Global Objectives

This is a collaborative study of dose-response relationships for cell transformation *in vitro* at as low a dose as can be achieved. A realistic minimum dose at which to assess transformation frequency with adequate precision and achieve meaningful comparisons between participating laboratories is likely to be 0.1 Gy. Close links are maintained with other laboratories working on cell transformation and with groups developing epithelial systems of human origin suitable for cell transformation *in vitro*.

Specific objectives include:

(a) the preparation of a standard manual for cell transformation assays; this will include precise criteria for scoring transformed foci and an explanation of the classification in terms of examples of transformed and non-transformed foci;

(b) intercomparison experiments at doses down to 0.1 Gy of X-radiation;

(c) ultimately, by pooling data from each individual laboratory, the determination of dose-response relationships at doses less than 0.1 Gy;

(d) the eventual use of epithelial cell systems and assays that have an increased relevance to human risk estimation; and

(e) the formation of individual subgroups for more specialised investigations: dose-rate effects with densely ionising radiations, the role of neighbouring entities of high ionisation density using ultrasoft X-rays and Auger-emitters, transformation frequency and the cell cycle, alternative indicators of transformation and alternative transformation assays.

I.2 Global Achievements

I.2.1 Introduction

The principal risk from low doses of radiation is the induction of cancer. Currently the risks of developing cancer are predicted by various methods but these have not been validated at low radiation doses as routinely received during the operation of nuclear facilities and other sources of occupational exposure. Dose-response relationships for tumour induction can be studied using animal models but at low doses these become prohibitively expensive and anyway, may not be morally justifiable. An alternative is to use cell transformation *in vitro* for which a variety of systems are available. However only one, the C3H 10T½ mouse fibroblast system, provides the relatively high precision needed for work at low doses and dose-rates. This system is used in a number of laboratories in Europe and the USA.

It is clear that, if reliable data at low doses are to be obtained, a large number of transformants must be scored to reduce the statistical variation. For one laboratory this may put a large strain on resources. For such an internationally important topic, this seems an ideal area for collaboration between laboratories and in June 1990 a collaborative project, partially supported by the CEC (contract Bi7-043 B13), was initiated with the following objectives.

(1) To standardise the C3H 10T½ assay between participating laboratories.
(2) To produce a standard code of practice for cell transformation in C3H 10T½ cells; so that other laboratories may have a standard for the comparison of results.
(3) Ultimately, to establish the shape of the dose-response curve for cell transformation at low doses and dose-rates.
(4) To extend these studies to more relevant cell transformation systems (eg. human epithelial systems) as and when they become available.

The present contract is a continuation of contract Bi07-043 B13 with broadly the same objectives. In the report which follows collaborative intercomparison experiments carried out between September 1992 and August 1993 and involving all six participating laboratories are described in sections I.2.2 to I.2.5 below. This is followed by individual reports (sections II and III) from each participating laboratory.

I.2.2 Summary of Achievements under Contract Bi7-043 B13

The transformation assay, like many other biological assays, is susceptible to perturbations from a variety of influences. These perturbations do not normally hinder the comparison of results when made internally within each laboratory but often preclude the direct comparison of results between laboratories. Hence intercomparison of results between laboratories is difficult unless steps are taken to minimise the effects of these factors. Probably the most important factors for transformation are the criteria used for scoring transformed foci, the growth conditions (particularly serum batch) used for the assay and the source of cells used for experimentation. The initial phase of this project thus entailed the determination of the effect of the various factors on plating efficiency, cell inactivation and transformation frequency.

It was found that the most critical factor for cell transformation is the convention used for scoring transformed foci, hence several focus-scoring intercomparison have been carried out. For this purpose each group circulated a number of flasks or petri dishes for scoring by the other groups. This was followed up by a joint scoring exercise during which a consensus score for each flask or dish was determined. Altogether four such exercises have now been completed. Initially, there was a relatively large discrepancy between some laboratories (up to a factor of 2), but the disagreement following the third scoring exercise was reduced to a maximum of about 20%. It is clear that scoring is an influential parameter in the 10T½ transformation assay and regular joint scoring intercomparisons are necessary in order to maintain consistency between participating laboratories.

Other conclusions are (i) that the use of cells kept on ice for up to 72 hours does not compromise experiments, thus enabling the transportation of experimental cells between laboratories; (ii) that serum batch, as long as this has been previously screened as suitable for transformation, is not critical; and (iii) that the use of seeding densities between 1 and 3 viable cells per square centimetre is a region of small variation in transformation frequency and should be used for all routine experiments.

I.2.3 Focus Classification

As a basis for the consensus scoring, the criteria of Reznioff *et. al.* (Cancer Research, 33, 3239-3249, 1973) are used. Type I foci (foci containing tightly packed cells) are not scored as malignantly transformed. Type II (massive piling up into opaque multilayers; cells only moderately polar; criss-crossing not pronounced) and Type III (highly polar; fibroblastic; multilayered criss-crossed arrays of densely stained cells) are scored as malignantly transformed. The diameter of the foci is not considered as a relevant parameter. For the intercomparison of scoring no distinctions between type II and type III foci are made and the most important criterium is the presence of criss-crossing. Thus foci not scored as malignantly transformed are of the following morphologies:

(A) Foci containing tightly packed cells without massive piling up and without criss-crossing.
(B) Foci showing massive piling up into virtually opaque multilayers. These may be uniformly dense or corded in gross morphology; however, criss-crossing of highly polar cells is absent.

Foci scored as malignantly transformed have the following characteristic morphologies:

(C) Foci showing massive piling up into virtually opaque multilayers with pronounced highly polar, fibroblastic, multilayered criss-crossing at least at one site. Foci may be uniformly dense or corded in gross morphology.
(D) Foci composed of cords containing criss-crossing of highly polar cells without massive piling up within the cords.

In addition there is one other class of focus, designated type X, which, at present is not classified as being malignantly transformed due to the absence of criss-crossing.

(X) Foci characterised by long fibrous sheets with sometimes piling up along the strands but with no criss-crossing.

However, preliminary tumourigenicity studies suggest type X foci should be classified as being positively transformed (see below).

An important goal, which has now been achieved, is the production of a catalogue depicting various classes of foci and the score assigned to these foci. This is a unique reference for scoring foci and will be an aid to other laboratories using the C3H 10T½ cell transformation assay to whom it is made readily available.

I.2.4 Tumourigenicity Studies

In order to validate the scoring criteria as defined in section I.2.3, a number of foci have been isolated from culture and tested *in vivo* for tumour growth. The results of tests on fourteen foci are shown in table I.

TABLE I: Results of Tumourigenicity Studies

Focus Identification	Date Mice Injected	Tumour Status (1/9/93)	Flask Score	
			Berkeley	Consensus
3M X	26/2/93	1/3	X	X
3M A	23/2/93	4/4	X	+
3M C	23/2/93	3/3	X	X
3M E	23/2/93	4/5	X	X/+
1M B	23/2/93	2/4	+	-?
1M D	2/3/93	1/3	+	+
2M E	9/3/93	5/5	+	+
3M D	25/2/93	4/4	-	+
3M F	23/2/93	6/6	?	+
3M G	23/2/93	0/4	-	-?
3M H	9/3/93	5/5	?	+?
3M K	2/3/93	0/6	-	-
3M L	2/3/93	1/3	-	+
3M M	9/3/93	5/5	?	+
"Normal" 10T½ cells	26/2/93	0/2	NA	NA
No cells	23/2/93	0/4	NA	NA
No medium, no cells	1/3/93	0/5	NA	NA

For these tests foci were isolated from cultures irradiated at Berkeley with a dose of 5 Gy of X-rays. Cells were sent to St Andrews University where they were injected into athymic C3H mice. Prior to the tumourigenicity results being available the foci were scored, initially by Berkeley alone, and then by all laboratories. As can be seen from the table the correlation between focus score and tumourigenicity is excellent. The results also suggest that type X foci should be classified as positive.

Tumour cell lines have been established from some of these foci and chromosomal studies have been carried out at Harwell. The results of these studies are reported in section III. Further foci (spontaneous, X-ray and α-particle induced) are currently undergoing animal tests.

I.2.5 Survival and Transformation Intercomparison Experiments

Following the intercomparison transformation experiments which were carried out at an absorbed dose of 5 Gy in order to determine the influence of medium type and other parameters on the transformation frequency, further measurements have now been undertaken at doses down to 1 Gy. As before, cells from one participating laboratory (Berkeley) were irradiated and immediately placed on melting ice at 0°C along with a sample of unirradiated cells. These were then despatched to the other laboratories for subsequent culture. Samples kept at Berkeley were processed immediately after irradiation with a second set of samples kept on ice for about 48 hours before processing. A common protocol was adhered to within the limits of each laboratory. Altogether five experiments at doses of 1, 2 and 3 Gy have now been carried out. The results for both survival and transformation are shown in figures 1 and 2. Clearly the reproducibility between laboratories for transformation at the lower doses is disappointing, despite the fact

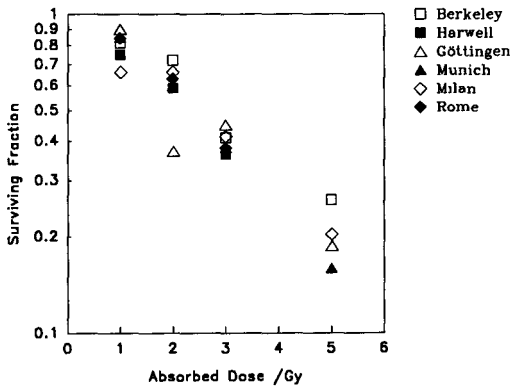

FIGURE 1: Intercomparison of Surviving Fraction

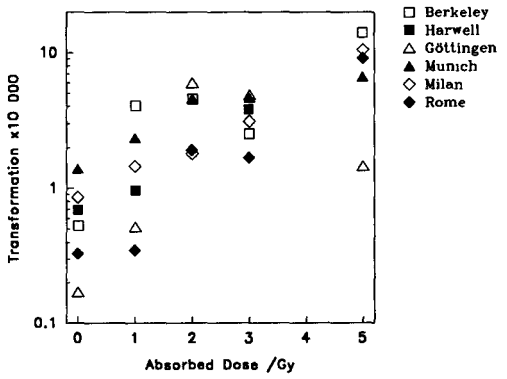

FIGURE 2: Intercomparison of Transformation Frequency

that each of the data points is an average of five separate determinations and each set of data was consensus-scored by all six participating laboratories.

Further investigations, however, have indicated that an important factor is the difference in culture conditions between the laboratories resulting in a significant spread in the time taken for confluence to be reached. Results from two intercomparison experiments in which the effect of incubation period was studied are shown in figure 3. Clearly there can be a large change in the measured transformation frequency between the standard period of six weeks and longer periods of eight or nine weeks. These results need to be interpreted in conjunction with growth-curve data for the same experiments which are summarised in table II. Evident is the spread in the time taken to reach confluence which seems to depend on serum batch and other factors peculiar to each laboratory. In additon to this laboratory variation it should be noted that 5 Gy-irradiated cultures can take up to a week longer to reach confluence than control samples despite being seeded at similar viable cell densities.

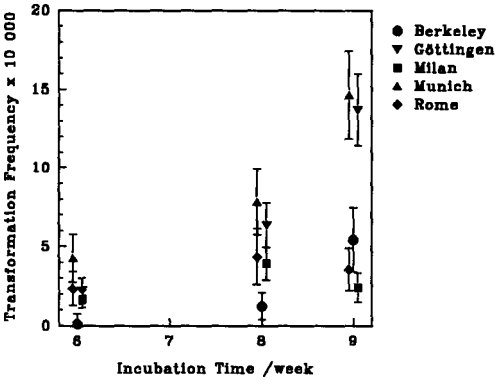

FIGURE 3: Transformation Frequency and Culture Time

TABLE II: Summary of Growth-Curve Data

Laboratory	Viable Cell Seeding Density /cm^{-2}		Approximate Cell Density at Confluence /10^4 cm^{-2}		Approximate Time to Reach Confluence /day	
	Control	5 Gy	Control	5 Gy	Control	5 Gy
Berkeley	1.6	1.3	3	2	18	28
Göttingen	1.7	1.5	6	4	17	21
Milan	2.7	2.7	5	4	15	18
Munich	2.3	1.7	5	4	17	21
Rome	2.0	2.4	4	4	17	19

The conclusion to be made from these studies is that the important time factor for the expression of transformation is the period for which the cultures are confluent. The optimum time appears to be five weeks. Future intercomparison experiments at doses down to 0.5 Gy are planned for October 1993 using this additional criterium.

Head of Project 1: Dr. Mill

II. Objectives for the reporting period

(a) Participation in the standardisation of the C3H 10T½ cell transformation assay.

(b) A study of dose-rate effects using 3.3 MeV α-particles and 250 kVp X-rays.

(c) Characterisation of transformed C3H 10T½ cells.

(d) Initiation of radiation studies using the human colorectal transformation system.

III. Progress achieved including publications

α-Particles - Dose-Rate Effects

The study of the effects of high-linear energy transfer (LET) α-particles is of importance for radiation protection as the human population is exposed to both natural and artificial alpha-emitting radionuclides in the environment. Of special relevance were the findings of Hill *et al.* (International Journal of Radiation Biology, 46, 11-16, 1984) on the greatly enhanced frequencies for oncogenic transformation in C3H 10T½ cells for very low dose-rates of fission neutrons. Data from our laboratory with neutrons and from elsewhere (Balcer-Kubiczek, *et al.*, International Journal of Radiation Biology, 59, 1477-1482, 1991) have not confirmed these initial findings. However, the controversy still exists as to whether irradiation by neutrons and α-particles at low dose-rates can be more effective than at high dose-rates. In particular, it has been suggested (Elkind, International Journal of Radiation Biology, 59, 1467-1475, 1991) that experimental artefacts could explain why many laboratories do not observe enhanced transformation rates at low doses of high-LET radiation.

We have cried out experiments using α-particles from a ^{238}Pu source for exponentially growing (log phase) and plateau phase C3H 10T½ cells cultured on Hostaphan based dishes (2.5μm thick). The α-particle energy was 3.3 MeV and the LET 121 keV μm^{-1}. A dose of 0.36 Gy was chosen for the dose-rate comparison. The dose-rates used were 0.18 Gy min^{-1} (acute), 1.2 mGy min^{-1} and 0.13 mGy min^{-1} (chronic), the lower dose-rates extending over irradiation periods of either 5 or 45 hours. The temperatures of the samples were maintained at 37°C throughout the irradiation period.

Rather than an enhancement our results suggest that, for log-phase cultures, there is a reduction of ~30% in the transformation frequency for the chronic dose-rates compared with the acute dose-rate. This is contrast to results reported by Miller *et al.* (Radiation Research, 124, S62-S68, 1990). This difference could be explained by the different techniques used in delivering the dose; whereas as Miller *et al.* used a fractionated regime, the results reported here are for continuous exposures resulting in a random sequence of events within the cell. We are currently carrying out a comparison between fractionated and continuous irradiations at 1.2 mGy min^{-1} in order to confirm this hypothesis which would indirectly support the existence of a sensitive period for transformation in the cell-cycle.

Because of a higher spontaneous transformation frequency for plateau-phase cultures no firm conclusions about possible dose-rate effects in these cultures can be made, although high

factors (*i.e.* >3) for an enhanced response at chronic dose-rates are not compatible with the observed data.

Optimisation of Transformation Assay

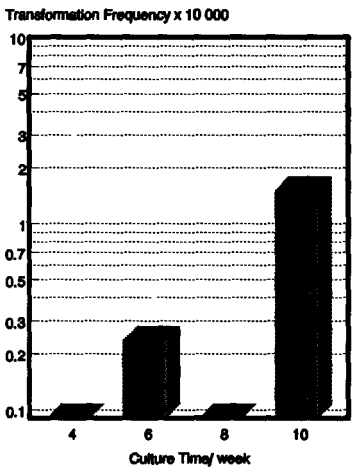

FIG. 1: The effect of culture time on transformation frequency (0 Gy)

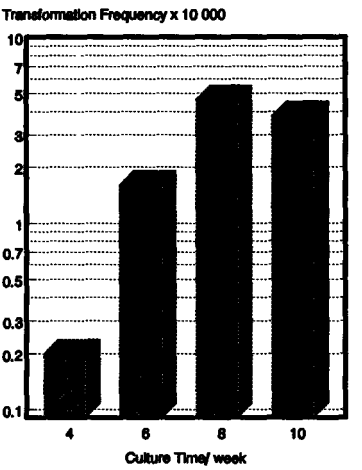

FIG. 2: The effect of culture time on transformation frequency (5 Gy)

Figures 1 and 2 show how the culture time affects the measured transformation frequency for C3H 10T½ cells; it is clear from these data that the conventional choice of six weeks for assessing transformation is not ideal and may be one of the reasons for the large differences which can exist between different laboratories using this assay. This, and other work on some characteristics (including tumourigenicity) of transformed C3H 10T½ cells has been reported in section I (summary of global objectives and achievements) of this report since this also forms an important input into the collaborative studies with the other six laboratories.

Other Studies

Recent work which has been initiated includes dose-rate effects for low-LET radiations and the introduction of the human colorectal transformation system.

ACKNOWLEDGEMENT

The α-particle irradiations were carried out in collaboration with the MRC Radiobiology Unit at Harwell. The MRC personnel involved in this work are: Dudley Goodhead, David Stevens and Samantha Marsden.

Head of Project 2: Prof. Dr. Frankenberg

II. Objectives for the reporting period

(a) Participation in the standardization and intercomparison experiments for oncogenic transformation of C3H 10T½ cells.

(b) Further experimental determinations of the oncogenic transformation in C3H 10T½ cells by carbon-K characteristic x-rays to confirm the preliminary results presented in the Final Report Contract Bi7-043 Sector: B13. Construction of an x-ray tube for the production off characteristic ultrasoft x-rays at high flux using electrons. This activity became necessary because the proton accelerator for the generation of ultrasoft x-rays is no longer available.

III. Progress achieved including publications

Characteristic carbon-K photons are an excellent tool for investigating the role of energy deposition patterns responsible for the induction of cancer, the principal risk from low doses of radiation. The photo-electron of a carbon-K photon has an energy of about 260 eV which generates 10 to 15 ionizations in a volume with a cord length of about 5 nm which is comparable with the diameter of the DNA double helix. The induction of DNA double strand breaks (DSB) by carbon-K photons is more effective by a factor of 3.2 compared with ^{60}Co gamma-rays (Frankenberg *et al.* 1986). Similarly, the RBE of carbon K photons at inducing chromosomal aberrations is approximately 3 (Virsik *et al.* 1980, Thacker *et al.* 1986). With this background, the RBE of carbon-K photons at inducing cell transformation was determined.

This report presents the final results for oncogenic transformation of C3H 10T½ cells after exposure to characteristic ultrasoft x-rays. By bombarding an extremely pure graphite target with 520 keV photons a carbon-K photon beam was obtained which traverses a monolayer of C3H 10T½ cells. The cell nucleus of these cells has a mean thickness of only 2 µm yielding an average dose, D, of 0.55 of the entrance dose. The cell survival as a function of the average dose, D, after exposure to carbon-K photons and ^{60}Co gamma-rays (as a reference radiation) together with RBE-values at different survival levels are shown in figure 1. The transformation frequencies per 10^4 viable cells are presented in figure 2. The RBE-value for transformation is found to be dose-independent and amounts to 2.5. This RBE-value is in good agreement with the RBE-value of 3.2 observed for DSB induction and also with the RBE-value of around 3 found for chromosomal aberrations. This agreement of RBE-values supports the view that highly localized (5 nm) energy deposition events (278 eV) can efficiently induce DSB and chromosomal aberrations which may lead to oncogenic cell tranformation. Furthermore, by comparing the energy deposition pattern of carbon-K photons and ^{60}Co gamma-rays it is the electron track ends (electrons with energies between about 100 eV and several hundred eV) which are responsible for oncogenic cell transformation after exposure to gamma-, x- or electron-rays. Since the 520 keV proton beam is no longer available a special device consisting of an x-ray tube for the production of characteristic ultrasoft x-rays by electrons is in construction. In order to obtain a high flux the cathode is "transparent", *i.e.* it consists of a thoriated tungsten wire (diameter: 0.25 mm) which is spread in a planar "zigzag". The distance between the parallel-arranged electrodes can be minimized to about 5 mm in order to avoid space charge effects.

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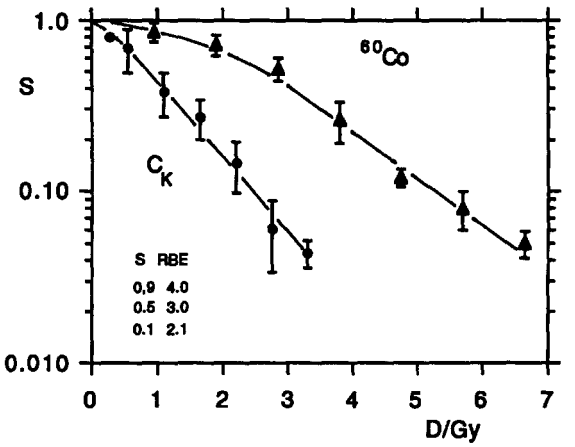

FIGURE 1: Final results for cell survival of C3H10T½ cells after exposure to carbon-K characteristic ultrasoft x-rays and ^{60}Co gamma-rays. S: cell survival, D: average dose.

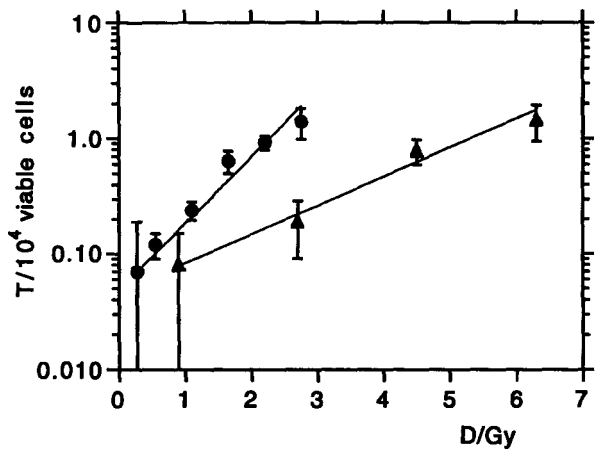

FIGURE 2: Final results for oncogenic transformation of C3H10T½ cells by carbon-K photons and ^{60}Co gamma-rays. T: transformation frequency, D: average dose.

Head of Project 3: Mr. Roberts

II. Objectives for the reporting period

This part of the programme aims to evaluate alternative or novel transformation assays. We have focused on two areas:

1. Evolution of the 10T½ assay by identifying markers of transformation
2. Characterisation of a transformation assay based on rat tracheal epithelial cells

Within area 1 the objective is to identify objective criteria for recognising transformed cells, improving the reliability and sensitivity of the technique. In addition it would be useful if the assay could be made quicker. The objective for area 2 is to evaluate the suitability of epithelial cells from rat trachea for either a quantitative transformation assay or as a 'bridging' system, allowing in vivo and in vitro results to be compared.

III. Progress achieved including publications

Area 1.

Representative foci from several transformation experiments were provided by Berkeley Technology Centre (BTC) for cytogenetic analysis. Following isolation, the foci had been expanded in culture and inoculated in nu/nu mice for confirmation of tumorigenicity. Cell-lines were established from the tumours thus obtained and these were also provided for cytogenetic analysis. Populations of cells were banked in liquid N_2 during the initial expansion of the foci after isolation and after the corresponding tumour had been explanted, providing a resource for the temporal dependence of any cytogenetic changes to be analysed. Thus far, three foci and their corresponding tumour-derived cell-lines have been analysed and compared with the original 10T½ parent line. The foci and tumour-derived lines appear to have lost chromosomes. The modal chromosome frequency differs for each cell line but, in general, the tumour-derived lines have fewer chromosomes than lines from the corresponding focus, which in turn have fewer than the parent line. One possible explanation for this is that the focus-derived cell line contains a substantial number of non-transformed cells which are lost on passage through an animal. All the cell-lines examined so far have a distinctive, large marker chromosome, probably the result of a Robertsonian fusion. The identity of the chromosomes involved in the fusion has yet to be determined.

Area 2.

The RTE assay has several features that make it worth investigating. Firstly, it is based on a relevant epithilial cell type; secondly, the cells may be treated before or after isolation (a particular advantage for studies on radon); thirdly, transformation may be assayed in vitro or in vivo, making possible validation of the in vitro measurements and finally both in vitro and in vivo measurements can be compared with actual carcinogenesis. Furthermore, the use of transgenic animals could allow an informative comparison between mutation and transformation frequencies.

The RTE assay has a number of applications in radiological protection, however, it essential that the assay is properly characterised at the outset before any quantitative measurements are

undertaken so that any constraints on their use for transformation studies can be properly defined. Thus, preliminary studies with rat tracheal epithilial (RTE) cells have concentrated on characterising factors which influence their growth in vitro. Isolation procedures giving reproducible cell yields have been established and various factors affecting the plating efficiency (PE) of the cells identified. Among these are: age of the donor animal, 7 - 8 weeks being significantly better than 11 - 12 weeks; use of feeder cells, at present we find that 3T3 cells improve the reproducibility but do not necessarily increase the PE; source of bovine pituitary extract, an essential growth factor in the serum-free medium; and conditions for enzymatic isolation of the cells, we routinely obtain < 1% PE with an overnight digestion at 4°C and now use 37°C for 45 min.

However, while it is possible to culture the RTE cells routinely, achieving a reproducible PE has proved more difficult. Current values range from 1 - 8%. A further difficulty with the technique is that several different cell types are isolated. The trachea has a pseudo-stratified epithilium containing a variety of different cell types. It has been suggested that although a number of cell-types are released by the enzyme treatment, only one, the basal cell, can grow in culture. Attempts to improve the PE by purifying the basal cells using magnetic separation techniques have not been successful.

IV. Objectives for the next reporting period

Area 1.

The programme of screening cell lines derived from foci and corresponding tumours will be continued. Also, cell fusion experiments will be undertaken to establish whether complementation groups exist for tumorigenicity. Should this be the case it may provide a handle for isolating the genes involved and production of informative probes for transformation in a relatively straightforward manner. Such probes could be a useful tool for rapid and objective analysis of transformation in a given population of 10T½ cells and for determining whether similar genes are involved in other cell types. In addition, if the marker chromosome identified in preliminary studies is present in the majority of the cell lines the chromosomes involved will be identified by appropriate cytogenetic techniques

Area 2.

Attention will focus on:

1. The influence of initial plating conditions on subsequent growth of transformants.
2. Optimisation of growth conditions for transformants
3. Characterisation of the isolated cells with a view to purifying the cells that can be cultured.

Head of Project 4: Prof. Tallone Lombardi

II. Objectives for the reporting period

Study of transformation sensitivity in the cell cycle. One of the most promising of the several interpretations formulated to explain the inverse dose-rate effect for medium-high LET radiation is the notion that postulates the existence of a period of high sensitivity to transformation during the cell cycle.

We have planned a systematic study of the transformation frequencies induced in synchronized C3H 10T½ populations in various phases of the cell cycle by low doses of 4.3 MeV, LET=101 keV/μm α particles.

An essential requirement for this type of experiment is to dispose of well synchronized populations which have the same characteristics as the exponentially growing populations. Our first goal was then to test different synchronization techniques to single out the most suitable in our experimental conditions.

The next objective is to irradiate synchronized cells with 7 cGy and 30 cGy of 4.3 MeV α particles at various times during the whole cell cycle. Three experiments have already been performed at 7 cGy and very preliminary results are now available. Experiments at 30 cGy are planned for the next few months. The results will then be analyzed in the light of those already obtained from fractionation experiments.

III. Progress achieved including publications

Many of the chemicals used to synchronize cell populations are known to be highly oncogenic. To avoid such problems we used synchronized populations produced either by releasing cells from contact inhibition confluence or by mitotic shake-off.

a) Release from confluence. Cell confluence was obtained following two different protocols. The first one was described by Terasima *et al.*: cells were plated at a density of 2.7×10^3 cells/cm^2 in 10 cm diameter dishes and allowed to grow for 11 days with a medium change on the 6th day. The second was described by Grisham *et al.*: cells were plated at a higher density (1.8×10^4 cells/cm^2) and allowed to grow for 4 days. In both cases cells were replated at a lower density in purpose build dishes with a mylar bottom suitable for α irradiation.

High yields of cells were obtained with these techniques: about 2×10^6 per 10 cm diameter dish. Progression of the population through the cell cycle after replating at low density was studied by flow cytometric analysis. Initial synchronization was quite good, 70-80% were G1 cells, but more than 50% remained in G1 over the period of the experiment. The doubling time of the population was more than 25 h compared with the value of 18-20 h for the asynchronous population. With the first method about 20% of the population showed a double DNA content at the time of the release. Furthermore in some experiments the spontaneous transformation frequency was very high, about 10 times the historical background. Our conclusions are that, although these two methods provide high yields of cells and good initial synchronization, they are not suitable for transformation assay.

b) Mitotic collection. Following the protocol of Miller *et al.*, one day before the collection, $8x10^5$ cells were plated in 150 cm^2 flasks (50-60 per experiment). The flasks were then struck firmly to dislodge rounded poorly attached mitotic cells. Three collections were performed, the first was discarded, the second placed in an iced bath for 1 h. A total of $40x10^3$ cells/flask were collected. Mitotic collection results in relatively low yields so that progression through the cell cycle was monitored by measurements of mitotic index, number of paired G1 cells and DNA synthesis, flow cytometric requiring too high a number of cells.

Mitotic indices at the time of mitotic shake-off were 70-80%. When cells were examined 2 h after plating of mitosis, it was found that the number of paired G1 cells was approximately 75% and the mitotic index had declined to about 0.2%.

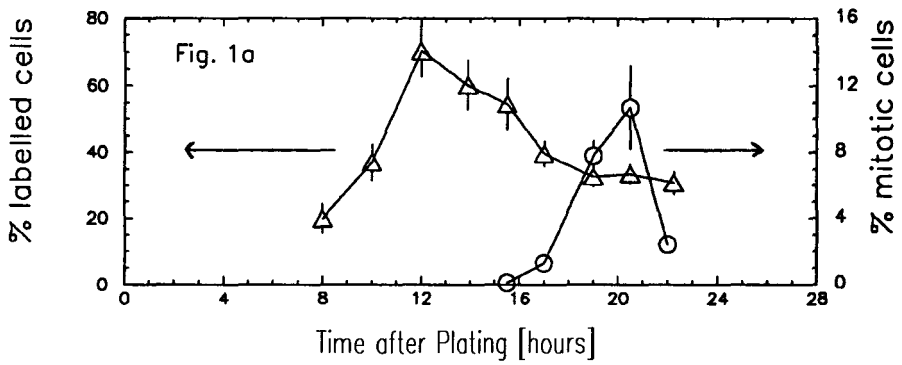

Fig. 1a shows the fraction of population synthesizing DNA (left scale) and the values of the mitotic index (right scale). The percentage of ^{3}H-thymidine labelled cells reached the maximum value of 70% at 12 h after the collection. The percentage of mitotic cells increases from 0.1% at 15.5 h to a value of 10.7% at 20.5 h. The most probable generation time was 20.5 h, similar to the value of the asynchronous population.

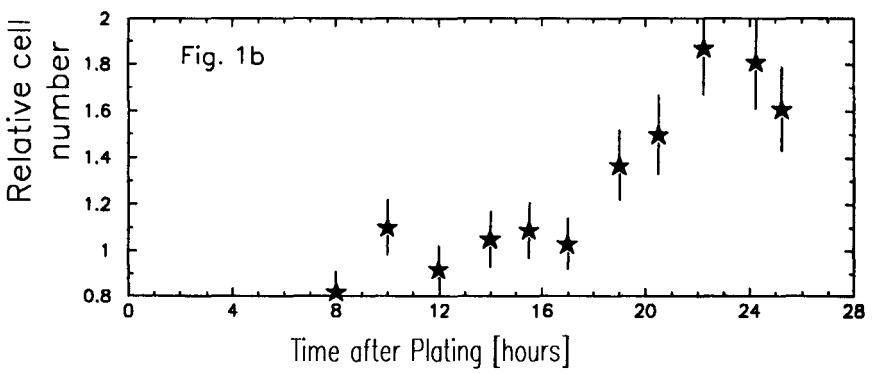

Fig. 1b shows the growth curve for the same population: there is a region of plateau between

10 and 17 h followed by a region between 17 and 22 h where cell density increased from 1.08±0.07 to 1.84±0.14. Division occurs between 17 and 22 h. Generation times observed in 3 experiments were between 20.5 and 22 h.

Similar results were obtained in two other experiments where, following a suggestion by C.K. Hill, two collections only were performed. The first was discarded and the second used immediately so that maintenance in ice was avoided. Cells were plated ($3x10^5$ per flask) 85 h before the collection. In both cases mitotic index peaked earlier: at 17 and 19 h with a value of 20% and 27% respectively.

Our conclusion is that the shake-off method, with a time interval of 3 days between plating and mitotic collection and no maintenance in ice, seems to be the best technique to obtain synchronized populations suitable for transformation assay.

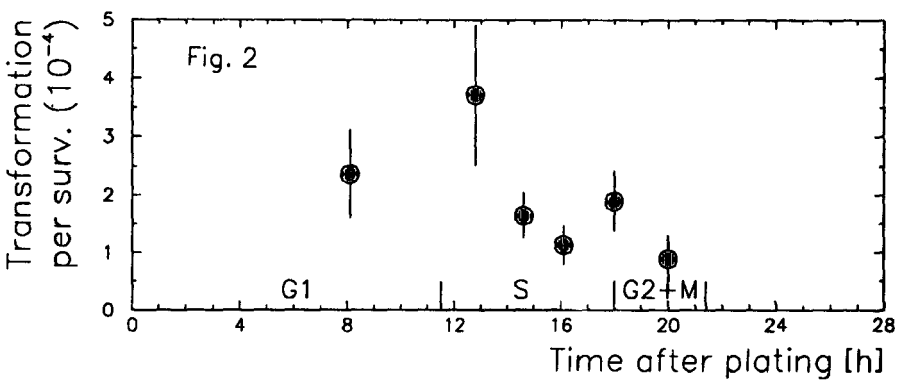

Fig. 2 shows very preliminary results on transformation frequency through the cell cycle after 7 cGy of 10 1 keV/μm α particles. The data are the results of about 2000 dishes and a total number of foci of about 70. Statistics need to be improved. The data available at the present seem to indicate that late S is a region of resistance for transformation. Indeed the frequency of transformation is similar to the spontaneous level. On the other hand late G1/early S seems to be a more sensitive region for transformation.

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Head of Project 5: Prof.Dr.Kellerer and Dr.Hieber

II. Objectives for the reporting period

(a) Participation in the standardization of the C3H 10T½ transformation assay to measure the transformation frequencies at low doses of sparsely and densely ionizing radiation.

(b) Investigation of cellular, cytogenetic, and molecular mechanisms underlying radiation-induced cell transformation.

III. Progress achieved including publications

Syrian Hamster Embryo (SHE) cells are a powerful *in vitro* model system for studying the process of neoplastic transformation since they can be transformed by various agents. Cells of transformed phenotype induce tumors in nude mice after a short latency period, and tumor cell lines can be established. In addition, SHE cells do also immortalize spontaneously but at very low frequency. These spontaneously immortalized cell lines are non-tumorigenic, but they became neoplastically transformed if after 40 or more subcultures. Therefore, several stages in the transformation process can be studied.

In the previous report (contract BI7-0043), we have shown that the expression of several oncogenes is increased in radiation-transformed SHE cell lines. In addition, preliminary results on the deregulation of several other genes that appear to be involved in neoplastic transformation have been presented for one tumor cell line. During the last year we have extended this analysis to a number of cell lines that have been immortalized and transformed either spontaneously or by alpha-particles or by 8 MeV/u carbon ions. The expression of particular genes were studied by Northern Blot analysis. The up- or down-regulation of the vimentin gene, the laminin receptor gene, type IV collagenase gene and the thymosin ß4 gene are summarized in fig.1. The laminin receptor gene, the type IV collagenase and thymosin ß4 especially are highly overexpressed in spontaneously immortalized SHE cells and in the tumor cell line T2655, established from a subsequently transformed cell line. However, radiation-transformed cell lines also show such over-expression, but to a lower extent. The tumor cell lines exhibit only moderate changes in the expression of these genes. All the genes studied have been shown to be involved in animal or human carcinogenesis. At present, we are studying the mode of derugulation. Changes in gene regulation might be due to translocation of the genes. This will be studied by fluorescence *in situ* hybridization (FISH) analysis.

Based on earlier data for cytogenetic changes in radiation-transformed and spontaneously immortalized SHE-cells, we have extended the investigations of specific chromosome alterations in radiation-transformed SHE cells. The deletion in the short arm of chromosome #2 (2p-) that has been found in spontaneously immortalized cells is also present in several transformed cell lines that have been exposed to carbon ions. However, the data suggest that this deletion occurs spontaneously and it is not likely to be induced by radiation. The data are summarized in table 1.

Furthermore, additional individual cell lines, transformed by gamma rays, have been analysed for chromosome abnormalities. Six out of eight cell lines show a common 3q+ and 6q+ aberration, *i.e.* there is additional unidentified chromatin at chromosome #3 and #6 (see fig.2). These abnormalities are also found in most of the transformed primary colony. Whereas 2p-

appears to occur spontaneously, the 3q+ and 6q+ seems to be radiation-induced. The genetic material has now to be identified and the functional importance of these cytogenetic changes for the transformation process needs to be analysed.

PUBLICATIONS

J.Smida, L.Hieber, and A.M.Kellerer (Abstract): Increased expression of a laminin-receptor gene in tumor cells derived from radiation-transformed Syrian Hamster embryo cells. Joint meeting of the Association for Radiation Research, The Netherlands Radiobiology Society and The Swedish Radiobiology Society, St. Andrews, UK, 1.-4.4.1992. Int.J.Radiat.Biol., 1992, 62, No.3, 378-379

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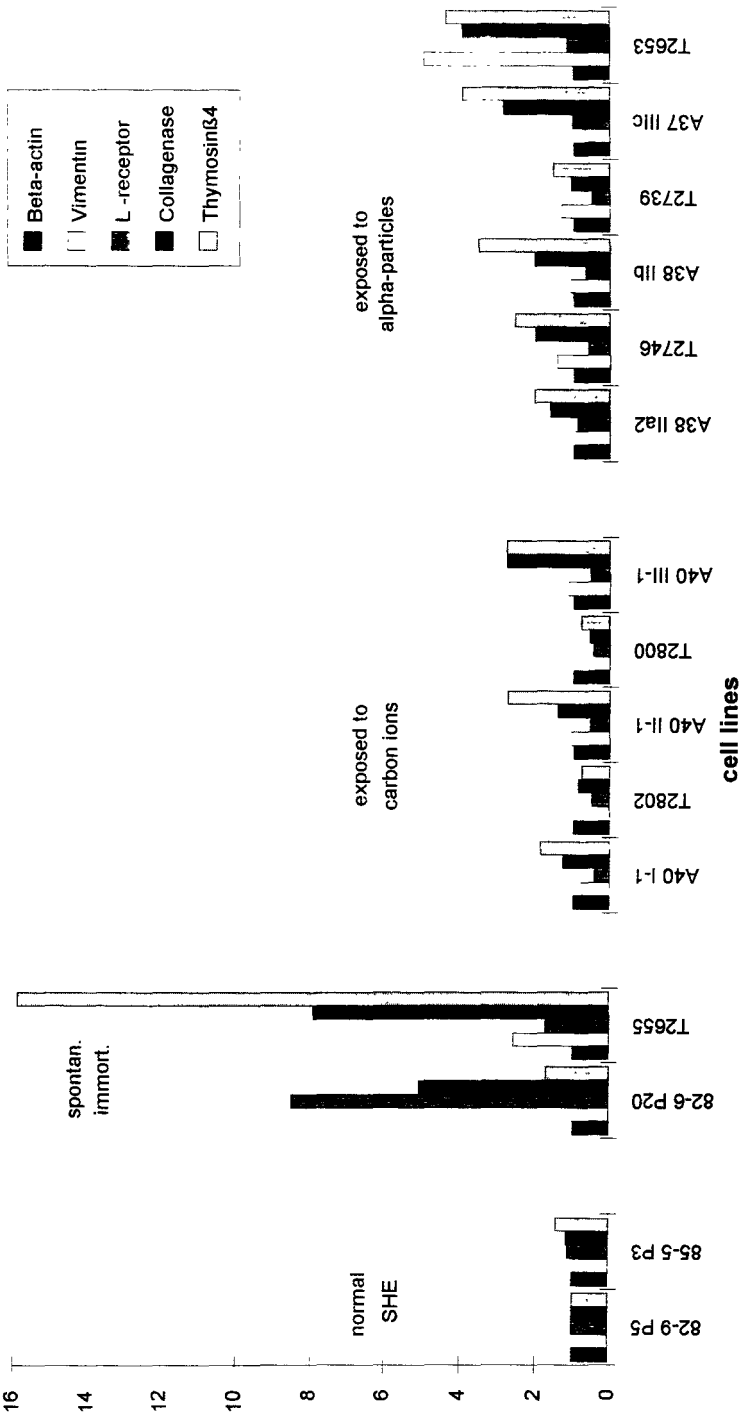

Figure 1: Expression pattern of different genes in spontaneously immortalized (82-6 P20), in radiation-transformed SHE cells(A####) and in tumor cell lines (T####) compared with normal SHE cells (82-9, 85-5). All data were normalized to ß-actin.

Table 1: Cytogenetic changes in spontaneously transformed SHE cells

Cell Line	Passage No.	Modal Chromos. No.	Common Chromosome Abnormalities	Recurrent Chromosome Abnormalities
82-6	P4 normal	44	-	-
	P15	44	-	2p-
	P18	44	2p-	-13, -16
	P21	44	2p-	
	P56	44	2p-	
	T2340	44	2p-	+19
	P86	42/43	2p-,-12	
	T2283	45/46	-5, +8, -Y 2p-, +t(11q:12q) +t(5q;2q), +M	+9, -12, -13, +14 +i(19q)

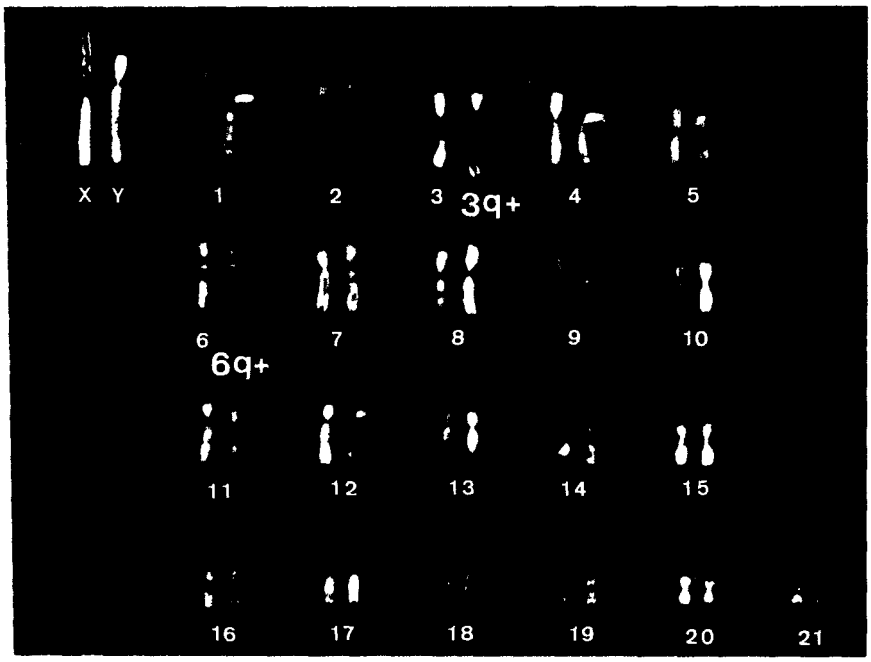

Figure 2: Quinacrine-banded karyotype showing a 44, XY, 3q+, 6q+ of a gamma-ray transformed cell line (primary colony).

Head of project 6: Dr. Saran

II. Objectives for the reporting period

- Investigate dose-rate and fractionation effects for neutrons at low doses and dose-rates
- Investigate the influence of the cell cycle on cell transformation
- Consider the suitability of a human epithelial cell line for radiation-induced transformation
- Maintain links with other groups developing human cell lines for radiation-induced cell transformation studies

III. Progress achieved inclusing publications

The contribution of the laboratory of carcinogenesis at ENEA-Casaccia Centre for the reporting period consists in dose-protraction studies with neutrons of different qualities on the C3H 10T½ cell transformation system; investigation of age-related sensitivity to neoplastic transformation after high-LET radiation. The use of human cell systems for transformation studies is being considered.

The participant laboratory examined the influence of dose fractionation of monoenergetic neutrons on neoplastic transformation of C3H 10T½ cells. Neutrons of 0.5, 1.0 and 6.0 MeV were utilised. Cells were exposed to doses of 25 and 50 cGy, given acutely or in 5 fraction at 2-hour intervals. Within the experimental errors, there was no difference in transformation frequency between acute and fractionated irradiation at all energies and doses tested. Transformation data were also analysed using a linear dose-effect relationship (P>0.4). There was no significant difference in the values of the transformation frequency per unit dose between acute and fractionated irradiation at all energies used, while there is a significant decreased effectiveness from 0.5 to 6.0 MeV neutrons, in both irradiation modalities. No significant dose-fractionation effect was observed on the survival of irradiated cells. These results are in agreement with our earlier data with fission spectrum neutrons from the RSV-TAPIRO reactor.

Cell-cycle dependence of oncogenic transformation of C3H 10T½ cells is invoked as a possible explanation of the inverse dose-rate effect for neutrons, observed at some laboratories. In particular, Brenner and Hall have proposed that the largest enhancement of transformation frequency due to a cell-cycle dependent susceptibility should be seen in the period of mitosis. Qualitatively different results have been presented up to now for such a cell-cycle dependence after low-LET irradiation, while for high-LET radiation the data are still very sparse. To gain additional evidence, we have collected mitotic C3H 10T½ cell enriched suspensions by the shake-off procedure. For a better characterisation of our cell populations as a function of time, as cell synchrony tends to be lost quickly, the mitotic indices as a function of time after the mitotic collection has been measured. Data of Fig. 1 suggest that the same number of cells in the initially mitotic population is still found in the peak around 15 hours, but with a much larger time spread. After this, the mitotic index tends to decrease to a value approximating that of the asynchronous population. Immediately after shaking, suspended cells were irradiated with either 400 cGy of 250 kVp X rays, or 50 cGy of fission spectrum neutrons from the experimental fast reactor RSV-TAPIRO of CRE-Casaccia. Mitotic cells were also immediately plated for irradiation at a later time corresponding to S/G_2 phase *i.e.* 15 hours after shaking, and trypsinized before irradiation. G_2/M phase cells were also

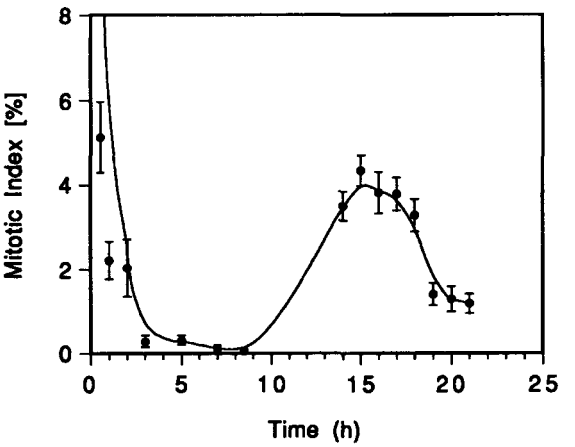

Fig. 1- Variations of mitotic index as a function of time after the mitotic collection.

examined by irradiating them with 400 cGy of X rays while they were still attached in flasks and dislodging them 25 minutes after the exposure. For comparison, asynchronous population of exponentially growing C3H 10T½ cells was irradiated with the same modalities. Our results, summarized in the Table, do not support the hypothesis that cells during G_2/M (Cao *et al.*, 1992) or S/G_2 (Miller *et al.*, 1992) phases are more sensitive to radiation induced neoplastic transformation. As far as neutron data are concerned the asynchronous population shows a transformation frequency double compared with the two phases of the cell cycle here examined in synchronous populations. This could mean that if there is a variation in sensitivity to neoplastic transformation this should probably be in G_1 or G_1/S period. This hypothesis needs further verification, and additional experiments are in progress.

NEOPLASTIC TRANSFORMATION OF ASYNCHRONOUS AND SYNCHRONOUS C3H 10T½ CELLS EXPOSED TO FISSION NEUTRONS AND 250-KVP X-RAYS.

Dose (cGy)	Time relative to mitosis	Surviving fraction	No of viable cells per dish	No of dishes Tot	with foci	Tr F /10^4 surv. cell	SE
Neutrons							
Asynchronous Cells							
0	-	1	269	762	2	0.098	0.069
50	-	0.455	248	752	18	0.977	0.229
Synchronous Cells							
0	0	1	232	991	1	0.044	0.044
50	0	0.445	256	1143	15	0.516	0.133
50	15 h	0.540	318	936	13	0.442	0.122
X-Rays							
Asynchronous Cells							
400	-	0.310	304	490	4	0.270	0.135
Synchronous Cells							
400	0	0.255	279	650	9	0.500	0.166
400	15 h	0 443	399	371	5	0.340	0 152
400	-25 min	0.213	209	139	1	0.345	0.345

We are using the human epithelial cell line RHEK-1(Rhim *et al.*, 1985) in order to test its ability to be developed into an assay system with which dose-response relationship for ionising radiation may be measured. Cellular growth parameters have already been determined. Experiments in progress are aimed to measure survival following high or low-LET radiation. Researches planned after summer 1993 concern the study of morphological alterations of the cells, and of abnormal patterns of growth after either fission spectrum neutron or X ray irradiation. For this purpose, we are considering the following endpoints (i) increased saturation density, (ii) growth in soft agar, (iii) focus formation.

PUBLICATIONS

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2. Di Majo, V., Rebessi, S., Coppola, M., Saran, A., Pazzaglia, S., Covelli, V. Are somatic effects of low neutron doses detectable in vivo? In Low Dose Irradiation and Biological Defense Mechanisms (T. Sugahara, L.A. Sagan, T. Aoyama, eds.), pp. 199-202. Elsevier Science Publishers B.V., 1992.
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4. Saran, A., Pazzaglia, S., Pariset, L., Coppola, M., Di Majo, V., Rebessi, S. and Covelli, V. Dose fractionation dependence of C3H 10T½ cell transformation by fission spectrum neutrons. 40th Annual Meeting of the Radiation Research Society. Salt Lake City, 1992.
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7. Saran, A., Pazzaglia, S., Pariset, L., Rebessi, S., Coppola, M., Di Majo, V., Covelli, V. Cell-cycle dependence of C3H 10T½ cell transformation by fission spectrum neutrons. 41th Congress of the Radiation Research Society. Dallas, March 1993.
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Progress Report

Contract: **FI3P-CT920053** **Sector: B13**

Title: Molecular and cellular effectiveness of charged particles (light and heavy ions) and neutrons.

1)	Moschini	INFN - Legnaro
2)	Michael	Hosp. Mount Vernon
3)	Goodhead	MRC
4)	Belli	ISS
5)	Sideris	NCSR "Demokritos"
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I. Summary of Project and Global Objectives and Achievements

It has been shown and confirmed by independent experiments, performed at different european laboratories, that low energy protons are more effective in inducing cell inactivation and mutation than other heavier charged particles (namely, alphas) in the 10 - 35 keV/μm LET region. Recently, deuteron beams have been used to extend the earlier proton LET range to higher LET values, studying the induction of cell inactivation.

It is therefore useful to complete this work and to extend it using heavier charged particles and fast neutrons to perform, where appropriate, direct comparison of the biological effectiveness of the different radiations and to provide data for a more reliable environmental and occupational risk assessment.

Moreover, until now, non-radiobiological data exist regarding the effectiveness of a single and few charged particles (proton or alpha) to inactivate a single cell or to induce genetic damage, which is of crucial importance for the environmental risk estimation. This project proposes to start such a kind of investigation by exploiting the experimental opportunity provided by the microbeam facilities.

As far as cellular endpoints such as survival, mutation and transformation is concerned, it is generaly acknowledged that these are not only the consequences of physical factors, such as energy deposition events, but also the expression of several biological factors. From this point of view the nuclear chromatin organization plays an important role in determining radiation sensitivity, affecting both the amount of initial damage and the accessibility of the repair enzymes to the damaged sites. The majority of the data available in the literature has been obtained using actively proliferating cells. However, in a tissue, the largest part of the cells are in different stages of differentiation, unable to replicate and owing differential structural organization of the genome, different metabolism and enzyme content (including those involved in the repair processes). For this reason, the use of human cells capable to undergo "in vitro" differentiation can represent an important tool for radiobiological studies. This kind of approach

allows to better clarify the basic mechanisms of radiation action and have practical implication in radioprotection and radiotherapy. In the present project it is planned to use both the actively proliferating cells and the human (differentiated and non-differentiated) cells and to perform, where appropriate, direct comparison.

The main objectives of the project are to:

1) measure the effectiveness of deuterons in inducing HPRT mutations, as a function of LET;
2) measure the effectiveness of $^3He^{++}$ and α-particles as well as of heavy ions like Li, B, C, N, O and P in inducing cell inactivation and mutation;
3) make direct comparison of the effects of protons/deuterons with $\alpha/^3He^{++}$ for similar LETs as well as of the investigated heavy ions, where appropriate;
4) start comparative experiments of the effectiveness of high and low dose rate irradiation with α-particles;
5) start measurements of the effectiveness of fast neutrons in inducing cell inactivation and mutation;
6) measure the initial yield of DNA dsb in cells irradiated with protons, deuterons and helium ions;
7) start experiments on the effectiveness of a single and few charged particle (proton or alpha) to inactivate a single cell or to induce malignancy;
8) set up a beam line for irradiation of cell monolayers with heavy ions;
9) set up appropriate irradiation facilities based on the use of microbeam systems;
10) make comparative experiments using actively proliferating cells and "in vitro" human cell culture (P3, TK6 and K562), irradiated with protons, deuterons and alphas in the same LET range;
11) develope several new assays to detect the distribution of lesions within clustered damage sites induced by radiation of various LET;
12) analyse the microscopic track structures of radiations both with Monte Carlo calculations and deterministic approaches, to seek features, which correlate with their observed biological effectiveness.

Head of project 1: Prof. G. Meschini

II. Objectives for reporting period

1 - Completion of the study on biological effectiveness of deuterons for V79 cell inactivation as a function of LET and comparison with protons of the same LET.
2 - Extension of the study on deuteron effectiveness for V79 cell mutation at the HPRT *locus* as a function of LET.
3 - Start of measurements of the initial DNA dsb yield in V79 cells irradiated with deuteron beams.
4 - Study of the performances of heavy ion radiobiological facility and start of radiobiological experiments.

Objectives for next reporting period

1 - Completion of the experiments on deuteron effectiveness for V79 cell mutation at HPRT *locus* as a function of LET and comparison with our previous protons data.
2 - Start comparative experiments on cell inactivation using ^{3}He and ^{4}He beams with different LET values.
3 - Execution comparative experiments with low energy α-particles radionuclide source.
4 - Study of the biological effectiveness of heavy ions for V79 cells inactivation.
5 - Perform single particles irradiation of V79 cells using protons and alphas at the microbeam facility.
6 - Beginning of experiments on fast neutron biological effectiveness.

III. Progress achieved including publications

(In collaboration with ISS group)

In the framework of our systematic study on the biological effectiveness of deuterons, we have completed the experiments on V79 cell inactivation using a LET value not yet studied, 57 keV/μm, and carried out further experiments in the LET range 13 - 48 keV/μm, to confirm our previous findings; furthermore we have carried out measurements of the mutation induction at the *locus* HPRT in the LET range 18 - 57 keV/μm. It has not possible to carry out experiments at higher LET values due to the limited residual range of the particles as regard to the cell monolayer thickness, considered about 7 μm.

Figure 1 shows the RBE-LET relationship for the inactivation of V79 cells irradiated with deuterons with all the LET values we have studied. The RBE-LET relationship for protons, as we have already found, is also shown for comparison.

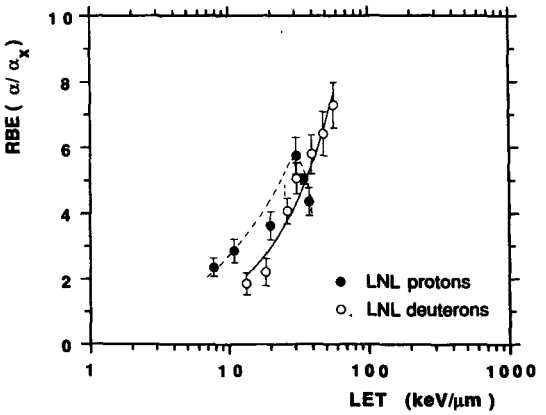

Fig. 1 : RBE-LET relationship for V79 cell inactivation

It can be seen that the RBEs for deuterons are lower than those obtained by us for protons of comparable LET in the range 13 - 31 keV/μm, while, at higher LET values, deuterons result more effective than protons. In the LET range studied the RBE-LET relationship for deuterons does not reach a maximum as protons show. Table I reports the RBE values for protons and deuterons evaluated as α/α_x ratio.

Table I: RBE values for cell inactivation

DEUTERONS		PROTONS	
LET (keV/μm)	$\alpha/\alpha_x \pm$ s.e.	LET (keV/μm)	$\alpha/\alpha_x \pm$ s.e.
13.4	1.87 ± 0.34	7.7	2.37 ± 0.28
18.4	2.23 ± 0.42	10.9	2.88 ± 0.36
26.3	4.09 ± 0.39	20.0	3.65 ± 0.43
30.8	5.08 ± 0.47	30.5	5.77 ± 0.55
39.5	5.81 ± 0.59	34.6	5.06 ± 0.15
48.0	6.43 ± 0.67	37.8	4.37 ± 0.42
57.0	7.30 ± 0.69		

As far as mutation induction at the HPRT *locus* is concerned, preliminary results are reported in figure 2, where each data point represents the mean value of at least 3 independent experiments. Due to the limited number of experiments carried out, it is not yet possible to establish the equation that allows the best fitting of the data. For this reason, for the interpretation of our experimental data we have considered, at present, both linear and linear quadratic equations. As figure 2 shows, while the dose-response relationship for the 18 - 31 keV/μm seems to be linear, the dose-response curve for deuterons of 39 - 57 keV/μm seems to show a curvilinear trend. Each dose-response curve corresponding to different LET values is reported, in a very preliminary form, with two different kinds of best fit obtained by using the equations:

Linear $\rightarrow$ $M = \alpha D$ and Linear Quadratic $\rightarrow$ $M = \alpha D + \beta D^2$.

Table II reports the parameters of the fits performed with both the Linear (L) or Linear Quadratic (LQ) functions for all the deuteron energies studied.

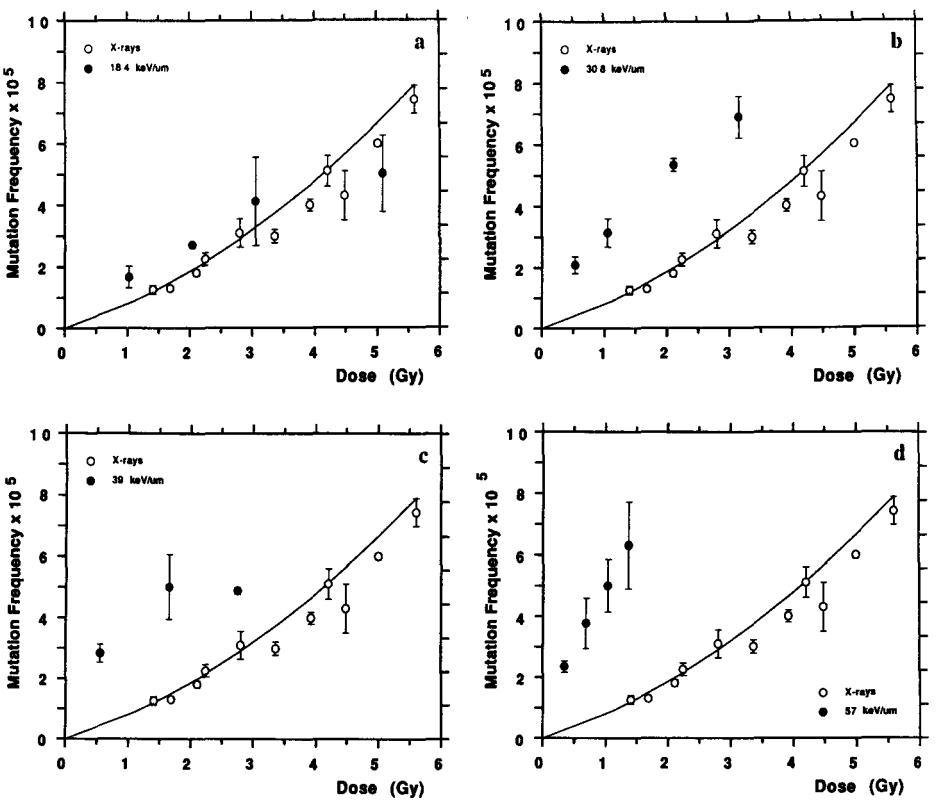

Fig. 2: Preliminary mutation induction data for V79 irradiated with deuterons at various LET: (a) 18.4 keV/μm, (b) 30.8 keV/μm, (c) 39.5 keV/μm, (d) 57.0 keV/μm

Table II: Parameters of the best fit for mutation induction dose-response curves

Deuterons	**Linear FIT** (L)		**Linear Quadratic FIT** (LQ)		
LET (keV/μm)	$\alpha \pm$ s.e. (GY^{-1})	RBE $\alpha/\alpha_X \pm$ s.e.	$\alpha \pm$ s.e. (GY^{-1})	$\beta \pm$ s.e. (GY^{-2})	RBE $\alpha/\alpha_X \pm$ s.e.
18.4	$(1.334 \pm 0.033) \times 10^{-5}$	2.2 ± 0.3	$(1.585 \pm 0.017) \times 10^{-5}$	$(-0.121 \pm 0.078) \times 10^{-5}$	2.6 ± 0.5
30.8	$(2.527 \pm 0.085) \times 10^{-5}$	4.2 ± 0.6	$(3.608 \pm 0.368) \times 10^{-5}$	$(-0.493 \pm 0.163) \times 10^{-5}$	6.0 ± 1.1
39.5	$(1.805 \pm 0.042) \times 10^{-5}$	3.0 ± 0.5	$(5.833 \pm 0.622) \times 10^{-5}$	$(-1.476 \pm 0.227) \times 10^{-5}$	9.7 ± 1.8
57.0	$(5.983 \pm 0.387) \times 10^{-5}$	1.0 ± 1.6	$(7.657 \pm 0.769) \times 10^{-5}$	$(-2.481 \pm 0.983) \times 10^{-5}$	12.8 ± 2.3
X-rays	–	–	$(0.60 \pm 0.09) \times 10^{-5}$	$(0.12 \pm 0.02) \times 10^{-5}$	1

In order to extend the study of lethal and mutagenic effects to other charged particles, such as heavy ions, on V79 cells, a dedicated irradiation facility has been installed and tested at the +60° LNL-XTU-Tandem accelerator beam line. Preliminary physical experiments have been performed using 140 MeV ^{58}Ni beam. It has not been possible to carry out biological determinations owing to technical setback of the machine accelerator. In the next scheduled calendar we will start this kind of experiments.

The "microbeam facility" at the LNL 2MV AN2000 Van de Graaff accelerator has already been installed successfully and is currently in operation essentially for elemental analysis. In order to study the biological effectiveness of charged particles in the ultimate limit of a single traversal, a single cell - single particle assay is under development. Biological experiments aimed to study V79 cell inactivation and HPRT mutation induction are planned to start within the present year.

In order to investigate a possible role of the dose-rate on the effectiveness of high LET radiations, we have planned to study the lethal and mutagenic effects in V79 cells irradiated with low dose-rate of source alpha-particles, in parallel with the experiments of irradiation of high dose-rate at the accelerators. Since irradiations at very low dose-rate, such as 0.005 - 0.01 Gy/min, require long time to deliver the established dose, this kind of experiments requires suitable conditions for continued cell growth, namely, controlled atmosphere with high umidity, elevated CO_2 tension and 37°C temperature to be carried out properly. For these reasons the irradiator used by us is of small size and especially designed to be put inside cellular colture incubator. For the preliminary irradiation we have used the alpha-source of ^{244}Cm, with diameter 25.4 mm, thickness 3.175 mm, active diameter 20 mm and activity 5 μCi supplied by ISOTOPE PRODUCTS LABORATORIES (USA). The alpha source energy of 5.8 MeV correspond to LET value of about 120 keV/μm at the cell surface. Physical and dosimetric characterization measurements have been performed. The biological experiments are planned for the present year.

Publications

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4) M. Belli, F. Cera, R. Cherubini, A.M.I. Haque, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron. Inactivation induced by low energy deuterons in V79 cells - S.I.R.R. - VI Convegno Nazionale, Capri, Italy, 19-22 October 1992, to be published in Physica Medica

5) R. Cherubini, F. Cera, A.M.I. Haque, P. Tiveron, G. Moschini, G. Simone, M. Belli, F. Ianzini, O. Sapora and M.A. Tabocchini. Mutation induction of low energy deuterons in V79 cells - Radiation Research society - 41st Annual Meeting, North American Hyperthermia Society 13th Annual Meeting, 1993 Dallas, Tx, USA

6) M. Belli, F. Cera, R. Cherubini, A.M.I. Haque, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron . Comparison between protons and deuterons with the same LET in inducing mutation in V79 cells - To be presented at the ESRB-93, Stockholm, Sweden, June 1993

7) M. Belli, F. Cera, R. Cherubini, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron . DNA double strand breaks induced by low energy protons in V79 cells - submitted to Int. J. Radiat. Biol. 1993

I. Partner: Cancer Research Campaign Gray Laboratory

Head of Project: Dr. B.D. Michael

II. Objectives for the reporting period

One of the objectives during the reporting period was to extend the earlier studies of ourselves and partners on the biological effectiveness of singly- and doubly-charged particles in respect of cell kill, DNA damage and its repair. This work was in part directed at providing useful constraints for studies modelling microscopic properties of radiation in relation to biological effect. A completely new arrangement for cell irradiation has now been developed to allow experiments to be done using protons and deuterons in an LET range partially overlapping that in which differences of effectiveness between these particles have been found by other partners. This arrangement will also allow us to make a direct comparison of the effectiveness of singly-charged ions (deuterons, in place of protons) with that of doubly-charged ions (helium-3, in place of α-particles) at near-matching LET's in the 60 keV μm^{-1} region, close to where we have observed peak effectiveness for protons/deuterons. Further work has also been done to determine DNA dsb induction and rejoining after proton and deuteron irradiation, assaying dsb by neutral filter elution, and has also been initiated using pulsed-field gel electrophoresis (CHEF). This forms part of an ongoing comparison of techniques for measuring dsb. These studies include the formation of more complex lesions, including the release of fragments after high-LET irradiation. We have also continued with the development of end-labelling assays for sites of clustered damage.

Development of our charged-particle microbeam facility has continued and pilot experiments with cells have been carried out. This facility, together with the one being developed at INFL Legnaro, will enable responses of cells to single particle tracks to be determined. This will allow our measurements of effectiveness of charged particles to be extended down to dose levels that mimic those that occur in protection-level exposures. In July 1993 we held a Gray Workshop "Microbeam Probes of Cellular Radiation Response", which was partially sponsored by the CEC Radiation Protection Research Action Programme, and was attended by groups from the EC, North America and Japan actively interested in these techniques and their uses in radiobiology.

During the reporting period we have continued several studies to elucidate early processes in the induction of DNA damage. We have obtained some initial data to determine the LET dependence of reactions of DNA radicals with a view to investigating the induction of clustered radical sites and the chemistry of complex DNA lesions. In other studies we have found a protective effect of DNA supercoiling against dsb induction indicating that DNA conformation influences OH radical attack. We have also obtained initial data in a study using synchrotron to determine the critical energies for dsb and ssb induction radiation which suggest that there is a common pathway for the induction of both lesions after direct energy absorption.

III. Progress achieved, including publications

Biological Effectiveness of Singly- and Doubly-Charged Particles for Molecular and Cellular Endpoints.

Cell survival

A new experimental arrangement for irradiating samples with low-energy singly- and doubly- charged particles is under construction. The new system has been designed to minimise, as far as possible, the energy lost by the particles reaching the cells. This will enable us to irradiate samples with ^{3}He ions of sufficient energy to match the highest practical LET's available from deuterons (around 60 keV μm^{-1}). It will also allow us to explore the effectiveness of protons and deuterons with LET's below 25 keV μm^{-1} and, in conjunction with Belli and co-workers, examine the unexpected difference in the RBE's of these two particles that they have reported for this LET region. A difference in effectiveness had not been expected between protons and deuterons of matched LET since they are believed to have almost identical track structures. Further work on this topic may, therefore have a significant bearing on our understanding of the role of track structure in radiation damage.

In the new arrangement, a gold scattering foil will be added to deflect a fraction of the particles through an arrangement of apertures and a vertical-slit exit window. The amount of radiation passing through the slit will be monitored by four encapsulated foils (two either side of the exit slit) operating as Faraday cups, within the vacuum. The rotating wheel that sweeps the samples across the slits can now be located just a few millimetres from the exit window so that the air path is minimised.

DNA damage

Previously we have shown that the yields of initial dsb measured using the neutral filter elution technique are similar for cells irradiated with singly or doubly charged particles and low-LET X-rays. This, we suggested, may be due to high-LET radiations inducing complex lesions in cellular DNA which current techniques may not distinguish at the molecular level. Further evidence suggesting that this is the case has been obtained from dsb repair studies where we have observed differences in rejoining of DNA dsb induced by 1.46 MeV deuterons and 0.74 MeV protons in comparison with X-rays. In particular, charged particle induced dsb are less likely to be rejoined than those induced by X-rays. Further studies are being carried out to measure the dependence of this difference in rejoining on LET and particle type as this may be an important indicator of the underlying lesion complexity induced by these radiations. In particular, the rejoining of dsb after irradiation with ^{3}He and ^{4}He will be determined. These studies have been carried out using the filter elution technique. We are also comparing the yield of dsb with these radiation types using pulsed-field electrophoresis techniques. These give the advantage that, under appropriate conditions, molecular weight information is obtained. A modified version of the clamped homogeneous electric field (CHEF) system is being used which allows good molecular weight separations in the 0.2 Mbp to > 5.7 Mbp size range after only a 48-hour electrophoresis run. Several studies have suggested that there may be some clustering of dsb induced by high-LET radiation which are related to the repeating units of higher-order DNA structure. An important use of the pulsed-field gel system will be to determine whether such clustering exists and what relationship it has to radiation quality and particle type. We are also developing molecular methods for the detection of

clustered lesions within small regions of DNA (1-20 bp). These involve end-labelling irradiated plasmid DNA molecules with ^{32}P using the enzyme polynucleotide kinase. The distribution of fragments produced can then be examined on 20% non-denaturing polyacrylamide gels. We have found this system particularly sensitive to the end-labelling of fragments sizes from the intact plasmid DNA molecule that we are using (4.3 kbp), down to fragments as small as 2-mer. Further studies will involve the pretreatment of DNA with other agents, known to convert other radiation-induced lesions to strand breaks, such as *E.coli* endonuclease III, S1 nuclease and alkaline pH treatment to determine the contribution of other lesions to clustered damage.

The Charged Particle Microbeam

With many aspects of the microbeam now operational, we have been able to begin pilot experiments (some involving the irradiation of cells) designed specifically to test the performance of the facility. All the electronic modules that interface the experiment to the computer are installed and working. Also operational is a mechanical shutter that uses a rotary solenoid and steel blades to block the beam within 50 ms of receiving the appropriate signal. This signal will eventually be generated from a detector between the exit of the final collimator and the cell (this is still under development). To detect the passage of protons we will use a thin (>10 μm) zinc sulphide scintillating crystal coupled to two fibre optics that pass light to two photomultiplier tubes operating in coincidence. The collimator is made from a glass capillary with an internal bore less than 10 μm. Using this collimator we have been successful in producing 'clean' (i.e. >95% close to full energy) protons collimated to within 10-15 μm. To produce finer collimators, we are considering other methods of manufacture.

The various micropositioning elements used to align the cells with the micro-collimator are now fully functioning under both manual and computer control. An X-Y-Z micropositioning stage is used to position the cells at the irradiation position and a motorised Z-drive attached to the final section of the beamline is used to bring the collimator rapidly to within a few microns of the base of the cell dish. The Z-drive is used to lower the collimator before each cell is located so that movement of the dish is not impeded. Prototype computer software for automating the experimental procedure is installed and in routine use. The co-ordinates of attached cells (stained with Hoechst 33258 DNA-binding dye and viewed using our solid-state fluorescence microscope) can now be determined and logged automatically using a software procedure. Currently, it takes about 20 minutes to raster-scan a 5 mm square region of the dish (about 100 screens). Continued use of this feature will enable the software to 'learn' how to distinguish cells from debris and other miscellaneous objects. Once the cell co-ordinates have been logged, it is possible to begin the irradiation sequence. The computer will automatically move the various micropositioning elements in the correct sequence and bring the first cell to the irradiation position. The cell is then irradiated by a single click of a computer 'mouse' (however, once the software is capable of distinguishing cells, even this operation will not be necessary). To preserve long-term registration between the computer co-ordinate system and the cell dish (even if parts of the microscope are re-aligned) a 'registration-plate' has been made. This plate locates in place of the cell dish and has two fluorescent 'corners' visible through the microscope. The computer can automatically find each corner and determine its exact position from a least-squares fit of the two edges defining the corner. The corner positions are used to convert the new position to a reference position. This calibration procedure is performed at the beginning of each session. One other

software feature has been installed to overcome the difficulty in aligning a cell dish that is inclined to the X-Y plane (this is almost inevitable on the micron scale). As such a dish is moved, the plane containing the cells will not stay in focus unless corrective measures are taken. Although an auto-focus facility is available, this would slow down operation considerably. Instead, the computer focuses on three different cells far enough apart to ascertain the plane that contains them. With this information, the cell dish is automatically maintained at the correct Z-position so that the cells stay in focus regardless of the X-Y position.

Several pilot experiments irradiating cells have been completed. Typically, these have involved identifying all cells in a 5 mm square area of the dish and irradiating each cell with about 1000 protons (sufficient to prevent all cells from forming colonies). After incubation of the dish we observe that the chosen area is indeed free of colonies, confirming that the alignment system is functioning properly (to within the spatial resolution of the collimator).

The chemical stage of radiation effect

We and others have made extensive studies of the chemical stage of radiation effect and this will contribute to the current aim of developing an integrated model linking the physical, chemical and biological stages. In common with others, our own work on the chemical stage has mainly employed low-LET radiation and, as a consequence, has tended to be dominated by single-radical chemistry, by default overlooking the multiple-radical chemistry that takes place at the important clustered damage sites referred to above. As described in the previous report, we have begun studies to enable us to follow the fast free-radical chemistry of clustered lesions in DNA. The methods we are developing are based on our ongoing work using fast-response techniques to measure reactions of thiols and oxygen with DNA radicals, but have now been adapted to the use of pulsed proton and deuteron irradiation, rather than the pulsed electrons used before. These techniques enable us to follow reactions of radical sites on DNA with a time resolution of about 100 microseconds. At such times after initial ionization, indirect effect damage by OH radicals will be complete and groupings of radical sites established on the DNA in accordance with the energy deposition patterns of the particle concerned. We anticipate that the chemical reactivity of these sites will reflect a signature of the spectrum of initial lesions. The system we have chosen to study initially is pBR322 plasmid DNA in the presence of GSH, because we have well characterized it in previous studies. Also, we are using the pBR system in other work on clustered damage, on the effects of DNA conformation and in measurements of the critical energies for DNA damage.

Data illustrating the determination of chemical repair kinetics of dsb precursor radicals by GSH are shown in Figure 1. In this example, damage has been induced by pulsed deuterons at an LET of 58 keV μm^{-1}. Experiments are in progress using protons and deuterons in the range 20 to 60 keV μm^{-1}, and the initial data show substantial LET-dependent changes in reaction kinetics which appear to reflect the proximity of DNA radicals induced by the tracks. The LET range used in this work matches that used in studies of DNA damage, RBE and OER in mammalian cells by ourselves and partners in the consortium. Together, these investigations will therefore be able to relate molecular endpoints as studied using these fast techniques to the effects seen in intact cells.

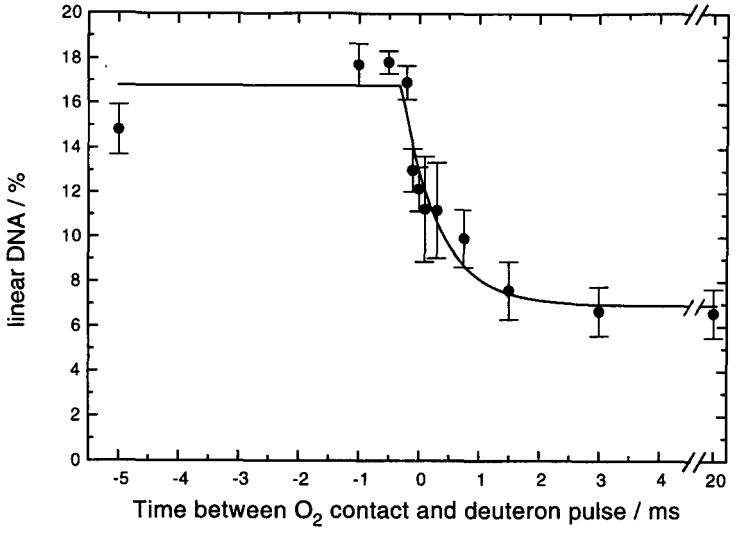

Figure 1 Fast kinetics of the oxygen effect on dsb in pBR322 plasmid DNA in the presence of 10 mmol dm^{-3} GSH, showing chemical repair of dsb precursor radicals induced by deuterons at 58 keV μm^{-1}.

Effects of DNA conformation on radiation response

As well as higher-order chromatin structure being an important determinant of cellular radiosensitivity, the degree of supercoiling of DNA may also be critical. For example, the accessibility of OH radicals and the binding of radioprotectors or radiosensitisers could be influenced by superhelicity. We have initiated studies using supercoiled plasmid DNA to investigate this possibility. The yields of DNA ssb and dsb after irradiation of plasmid solutions with pulsed electrons, which had different initial proportions of supercoiled molecules, has been determined. We have assumed that when plasmids are exposed to a single pulse of 1 microsecond duration this is much shorter than the time in which supercoiling relaxes. With an increasing amount of initially relaxed molecules, we find that the yields of ssb per gray remain similar. However, the yield of dsb increases, suggesting that relaxation of the supercoiled molecule allows greater accessibility to OH radicals. Thus it appears that in the supercoiled state, the intertwined duplex regions provide some protection to the each other against multiple damages involving OH attack.

Critical energies for DNA damage

This work is aimed at determining the relationships between the amount of energy deposited in DNA and the types and complexity of the lesions induced. The information so obtained is intended to provide insights into pathways for the induction of DNA damage. It is also intended to provide data useful for Monte Carlo studies modelling the interactions between particle tracks and DNA and the resultant lesions. Modelling and experimental studies have shown that, for a wide range of high-energy radiations, the most frequent energy depositions in DNA are around 25 eV, with an average energy deposition

somewhat higher than this value. Much of our work so far has therefore concentrated on energies below 100 eV, although we plan to include the somewhat higher energies thought to be important in the induction of clustered lesions. By using electrons and photons with well-defined low energies, it is intended to simulate selectively the wide range of energy depositions that occur with conventional high-energy radiations.

Our initial experiments studied the induction of ssb and dsb in pBR322 plasmids irradiated under dry conditions with electrons of defined low energies, using an evacuated diode type of irradiator, and a description of the methods and preliminary findings have been accepted for publication. We found that electrons with energies down to 25 eV were efficient at inducing ssb, but not dsb; however, we found that 50 eV electrons induced both types of lesion efficiently. This indicates that the threshold energy for dsb induction by electrons lies between 25 and 50 eV and is substantially higher than that for ssb induction. Electrons with such low energies have very short ranges, comparable with the diameter of the DNA itself. Consequently, our initial studies were complicated by shielding effects due to difficulties experienced in forming a uniform monolayer of plasmids. During the reporting period, we have developed much improved sample preparation techniques for the next phase of these studies.

We are also using monochromatic low-energy photons from the Daresbury synchrotron with similar objectives to those of the above electron work. Photons in the 10 to 100 eV energy range have the advantage that their penetration is about 10 times greater than that of electrons of similar energy, and we have found that the shielding effects referred to above are correspondingly reduced. Our work so far indicates that in the 25 eV region there is a substantial difference between photon- and electron-induced damage, i.e. that, unlike electrons, 25 eV photons are efficient at inducing dsb. The preliminary data shown in Figure 2 were taken early in the reporting period, before the above improvements in sample preparation had been made.

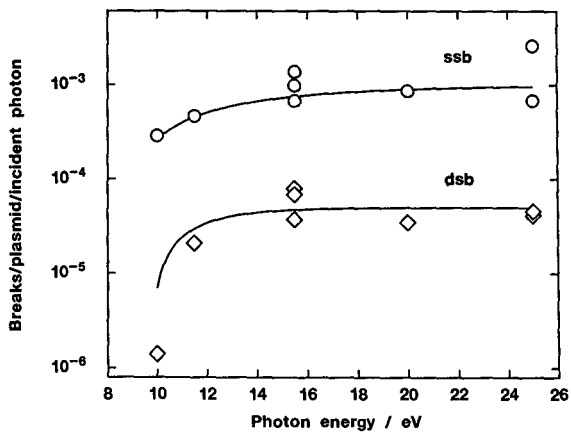

Figure 2 Spectral efficiencies for the induction of ssb and dsb in pBR322 plasmid DNA exposed to monochromatic synchrotron photons.

The data show that, as found by others, ssb are induced efficiently down to about 10 eV. However, they also show dsb induction down to about this energy, with a spectral efficiency approximately paralleling, on a logarithmic scale, that of ssb induction. It had previously been expected that, as with electrons, the threshold energy for dsb induction would be considerably higher than that for ssb induction. The apparent difference between the effects of the two radiations at about 25 eV may be related to the expected ratio of ionizations to excitations being greater for photons than for electrons of this energy. The approximately constant ratio of ssb to dsb (about 20:1) in Figure 2 suggests that the direct-effect damage induced by photons in this energy range may yield a common precursor of both types of lesion, with a 20-fold lower probability of generating a dsb than a ssb. Further work is being carried out using the improved techniques and, if the above preliminary results are confirmed, they may have significant implications for mechanisms of dsb induction and for modelling of DNA damage.

IV. Publications

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Head of project 3: D.T. Goodhead

II. Objectives for the reporting period

1. Comparison of dosimetric methods for charged particle irradiations at Legnaro and MRC.

2. Track structure analysis of proton and α-particle beams and comparison of their energy deposition patterns in nanometre volumes with their relative biological effectiveness.

Objectives for the next reporting period

1. Further analyses of CR39 track detectors exposed to particle beams at Legnaro.

2. Establishment of $HPRT^-$ mutation frequencies in V79 cells by slow α-particles (100-130 keV μm^{-1}) for comparison with results from other partners.

3. Extensive simulation and scoring of intermediate-LET α-particle tracks (~ 20-50 keV μm^{-1}) to establish stable results for these remarkably variable tracks.

III. Progress achieved, including publications

Others in this coordinated contract have previously shown, and confirmed, that the relative biological effectiveness (RBE) of protons rises rapidly in the region of 20-30 keV μm^{-1}, showing RBEs significantly higher than those of α-particles of the same LETs especially at the upper end [1]. These comparisons should provide a valuable probe of the biologically critical features of radiation tracks, which must in some way be responsible for the differences in biological effectiveness. Some of our effort has been aimed at trying to identify microscopic track features that may correlate with these observations. However, differences in experimental RBE between protons and deuterons of the same LET have also been reported and these could not be expected on the basis of current track structure simulations which assume that such particles of the same charge and velocity should have identical differential track structure [2]; thus there should be differences only in proportional energy loss, noticeable over relatively large distances. Our second contribution has therefore been to make some comparisons of dosimetric methods partially to test aspects of the experimental result.

In a limited comparison we applied our small-volume ionization chamber methods and CR39 track-etch detectors to a selection of proton and deuteron beams at Legnaro to compare with the dose rates and doses that the local team prescribed using totally independent dosimetric methods. The CR39 exposures in the position of biological samples also allowed evaluation of the uniformity of fluence across individual sample dishes and visualization of symmetry and size-uniformity of etched pits of individual tracks. The limited selection of beams tested accorded well with expectations on these criteria. Total fluence measurements on post-etched CR39 disks should be able to provide a reliable and independent method for evaluating absolute doses and comparing those from proton and

deuteron beams both in calibration tests and during routine biological experiments. Further CR39 samples need to be irradiated with the appropriate beams to allow these detailed evaluations that can be carried at a later time in another laboratory. Our 0.1 cm^3 thin-windowed ionization chamber also performed satisfactorily in these tests and can be used directly on line for comparative measurements of dose-rate and prescribed dose of proton and deuteron beams. Conversion to absolute doses would require local calibration checks of the picocoulomb meter in conjunction with the lengthy cables leading from the end of the beam line to the instrument position. Such detailed methods could be warranted to support specific findings of the CR39 comparisons.

To seek track properties that may correlate with the experimentally observed differences in RBE between protons and α-particles, we simulated many segments of tracks of these particles, using Monte Carlo code MOCA14 of Wilson and Paretzke, of LETs covering the range from about 20 to 40 keV μm^{-1} and also α-particles of higher LET up to more than 100 keV μm^{-1}. These were each scored for absolute frequencies of energy deposition, versus magnitude of energy deposition, in cylinders of diameters 2, 10 and 25 nm (and lengths ½ to 8 times the diameter) thrown randomly into the volumes containing all parts of each track. To date comparisons of any of these frequencies, as well as traditional parameters such as $\bar{z}_F$ and $\bar{z}_D$, has not revealed a property that simultaneously correlates well with the experimentally observed RBEs for both particles [2]. Although we could readily define parameters whose values for α-particles lay systematically below those of protons, the relative rise in α-particle values over the LET range 20-50 keV μm^{-1} had a general tendency to mimic the corresponding rise of the protons. However, this search for biologically relevant properties of the tracks is complicated by three factors: Firstly, there is not yet full experimental agreement between laboratories on the shape of the RBE-LET curve for protons particularly at the higher LETs and on where the RBE may maximize. Secondly, the experimentally reported difference between protons and deuterons is clearly beyond any property within the simulated tracks. Thirdly, the scored frequencies for α-particles at the lower LETs are remarkably unstable. Although we scored total track lengths, total numbers of ionizations and numbers of scoring cylinders similar to those that have given statistically stable results for other radiations these do not appear to be adequate for the α-particles [2]. Consequently, we need to increase the already large statistics and undertake repeat determinations on different samples of tracks to ensure stability of the results. This will be the next phase of our track structure work, while we await concordance of the experimental results within themselves.

Publications

[1] M. Belli, F. Cera, R. Cherubini, D.T. Goodhead, A.M. Haque, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini and P. Tiveron. "The importance of track structure of different charged particles having the same LET for biophysical modelling. In: Proc. Int. Conf. on Low Dose Irradiation and Biological Defence Mechanisms, Kyoto, Japan (in press, 1992).

[2] M. Belli, F. Cera, R. Cherubini, D.T. Goodhead, A.M.I. Haque, F. Ianzini, G. Moschini, H. Nikjoo, O. Sapora, G. Simone, D.L. Stevens, M.A. Tabocchini and P. Tiveron. "Inactivation induced by deuterons of various LET in V79 cells". Radiat. Prot. Dosim. (in press, 1993).

Head of project 4: Dr. M. Belli

I. Objectives for the reporting period.

1. Extension of the study on effectiveness of deuterons for inactivation of V79 cells as a function of LET.
2. Measure the effectiveness of deuterons for V79 cell mutation at the HPRT locus, as a function of LET.
3. Start studying the repair kinetics of molecular lesions.
4. Comparison of the effectiveness of high and low dose-rate irradiation with charged particles.
5. Use of human cellular systems.

II. Objectives for the next reporting period

1. Extention of the study on V79 cell inactivation and mutation induction to other particles such as alphas, heavy ions and neutrons.
2. Completion of the study on the effectiveness of deuterons for V79 cell mutation at the HPRT locus, as a function of LET.
3. Comparison of the effectiveness of high and low dose rate irradiation with charged particles in inducing lethality and mutation in V79 cells.
4. Measure of the initial DNA damage (dsb) and repair induced in V79 cells by different charged particles with comparable LET.
5. Extend the inactivation studies with high LET radiation, already performed on rodent cells, to human cellular systems.

III. Progress achieved including publications
(in collaboration with LNL)

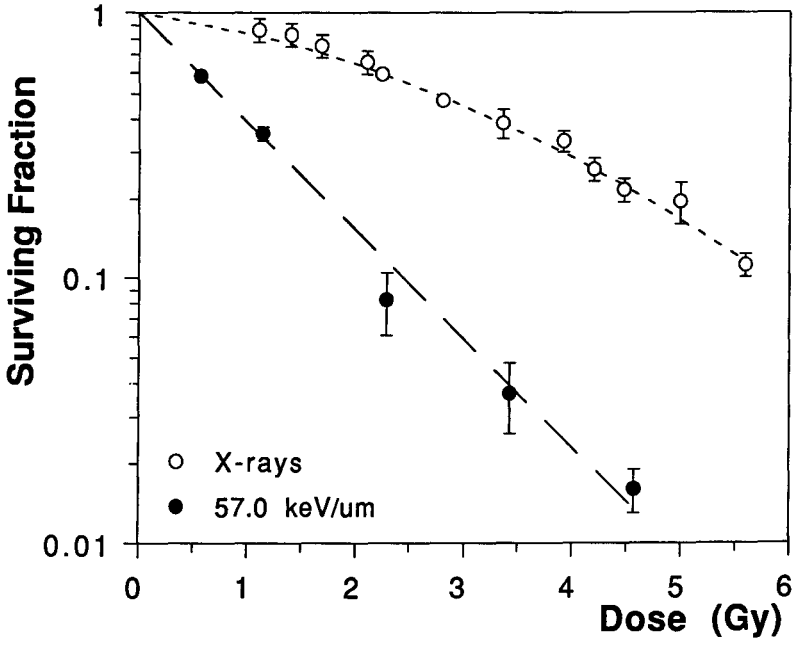
1. Our data on cell inactivation have already shown that there is an LET range where protons are more effective than alpha particles. More recently we found that the effectiveness of protons and deuterons is not the same at the same LET, even though they have the same charge and velocity. These findings point out that the RBE-LET relationships obtained with light ions may depend on the particle type (see previous CEC report) and prompted us to further extend the study to a wider deuteron LET range. Considering that the V79 cell thickness is 6 μm on average, as measured by confocal microscopy, the maximum LET suitable for our study is about 60 keV/μm (evaluated at cell midplane) in order to have a deuteron range longer than the cell thickness (Tab.I).

TABLE I

DEUTERON BEAM DATA

E_{beam} (MeV)	$E_{on\ cell}$ (MeV)	LET (keV/μm)	Range (μm)	$E_{at\ 3\mu m}$ (MeV)	$LET_{at\ 3\ \mu m}$ (keV/μm)	Range (μm)
3.82	0.76	49.9	11.0	0.62	57.0	8.3
3.90	0.97	42.0	15.6	0.80	48.0	11.7
4.00	1.21	35.9	21.8	1.06	39.5	17.8
4.20	1.63	29.2	34.7	1.51	30.8	30.7
4.40	2.00	25.3	48.2	1.90	26.3	44.1
5.20	3.20	16.1	107.1	3.16	18.4	103.1
6.50	4.92	13.3	217.7	4.87	13.4	213.8

The obtained dose-response curve for 57 keV/μm is shown in Fig.1 together with the curve for X-rays used as a reference for RBE evaluation, made in terms of linear coefficient ratio. This study shows that the RBE for deuterons monotonically increases with LET in the range 13-57 keV/μm, in contrast with the proton RBE-LET relationship that has a maximum at about 31 keV/μm (see LNL report).



2. Mutation induction of V79 cells at the HPRT locus after deuteron irradiation has been studied for particle energies of 5.2, 4.2, 4.0, and 3.8 MeV corresponding to LET values, evaluated at the cell midplane, of 18.4, 30.8, 39.5, and 57.0 keV/μm, respectively. The dose-response curves are reported in Fig.2 of LNL report.

Analysis of the data is still preliminary, being more experiments in progress. From the present data it is not clear whether deuterons give linear or sublinear responses.

Work is in progress to ascertain if the trend for the RBE-LET relationship is similar for both inactivation and mutation induction.

3. We have found that in the LET range 11-31 keV/μm the initial yield of dsb induced by protons, as measured by the low speed sedimentation technique, does not change significantly (see previous CEC report). This finding is in contrast with the results obtained for cell inactivation and mutation induction where a significant LET dependence has been found.

In order to get more information about the relation between molecular lesions and cellular effects, we have started experiments on the repair kinetics of damage induced by protons and on the measure, in the same LET range, of the initial yield of dsb induced by deuterons.

Completion of the data are planned for the next reporting periods.

4. In order to investigate a possible role of the dose-rate on the effectiveness of charged particles, an irradiation chamber has been developed for low dose-rate irradiation with an alpha source. The high dose-rate will be obtained using the accelerators at LNL. The chamber prototype, equipped with an alpha source of 244Cm, is already assembled at LNL and a second one is under construction to be used at the ISS. The biological experiments are planned for the next reporting period.

5. In order to perform experiments on human cells like TK6 and K562, that grow in suspension, an irradiation system has to be used different from that we used for irradiation of V79 cells that grow as monolayer. At present, we are testing different experimental set up to achieve this goal.

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1) M. Belli, F. Cera, R. Cherubini, A.M.I. Haque, F. Ianzini, G. Moschini, O. Sapora, G. Simone, M.A. Tabocchini, P. Tiveron. "Inactivation and mutation induction in V79 cells by low energy protons: re-evaluation of the results at the LNL facility". Int. J. Radiat. Biol., 63, 331-337, 1993.

2) F. Cera, R. Cherubini , A.M.I. Haque, G. Moschini, P. Tiveron, M. Belli, F. Ianzini, O. Sapora, M.A. Tabocchini, G. Simone. " Radiobiology and radiotherapy projects with accelerated charged particles at the INFN-Laboratori Nazionali di Legnaro: Present status and future perspectives". Physica Medica, 9, 161-165, 1993.

3) M. Belli, F. Cera, R. Cherubini, D.T. Goodhead, A.M.I. Haque, F. Ianzini, G. Moschini, H. Nikjoo, O. Sapora, G. Simone, D.L. Stevens, M.A. Tabocchini, P. Tiveron. "Inactivation induced by deuterons of various LET in V79 cells". Radiat. Prot. Dosim, in press.

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7) R. Cherubini, F. Cera, A.M.I. Haque, P. Tiveron, G. Moschini, G. Simone, M. Belli, F. Ianzini, O. Sapora, M.A. Tabocchini. "Mutation induction of low energy deuterons in V-79 cells" Forty first Annual Meeting of the Radiation Research Society and Thirteenth Annual Meeting of the North Amenrican Hyperthermia Society. Dallas, Texas, March 20-25, 1993, Abstract Book, P-25-6, p. 135.

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9) M. Belli. "Some applications of microbeam probes in basic radiation biology and biophysics". 4th L.H. Gray Workshop "Microbeam Probes of Cellular Radiation Response". Gray Laboratory, Northwood, U.K., July 8-10, 1993.

10) M. Belli, F. Ianzini, O. Sapora, M.A. Tabocchini, G. Simone, F. Cera, R. Cherubini, A.M.I. Haque, P. Tiveron, G. Moschini. "The RBE of protons for cell inactivation: the experience with V79 cells ". International Symposium on Hadron Therapy. Villa Olmo, Como, Italy, October 18-21, 1993.

Head of the project 5: Dr. E.G. Sideris

Scientific Staff: Ast. Prof. A. Anagnostopoulou-Konsta
Dr. C.A. Kalfas
Ast. Prof. G. Perris
Dr. G. Zarris

II. Objectives for the reporting period

Study of thermodynamic parameters following exposure of mammalian DNA to α particles and gamma rays and their relationship to biological parameters. Theoretical analysis of the results towards the development of a model describing the evolution of primary lesions induced on the DNA molecule.

During the next period the work will be continued on the same lines and will be expanded to cover measurements on changes induced by exposure to α particles on dielectric properties of DNA in comparison with our results which indicate the presence of dielectric changes during the helix to coil transition of gamma irradiated DNA, changes which precede those of base pair disruption.

III. Progress achieved including publications

(In text citations refer to the PUBLICATION LIST)

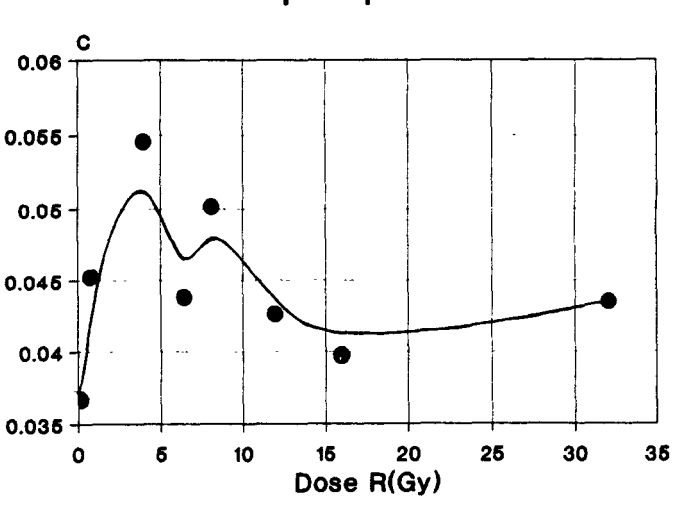
The presented work was designed under the assumption that most of radiation damage effected in vitro on DNA molecule is due to the induced water free radicals unless the DNA samples had been subjected to extremely low vacuum. Dielectric studies on mammalian DNA, conducted by our research group, have shown that there are 18-20 water molecules per nucleotide pair irrotationally bound to the DNA molecule forming a first hydration layer around the macromolecule . These water molecules can be removed only if the DNA samples will be subjected to low vacuum of the order of 10^{-5} to 10^{-6} Torr (Pilakouta, Sideris et al 1992).

The energy and radiation fluency in the samples were obtained from measurements taken with a silicon surface detector. The interactions with the water molecules were investigated using a semi-empirical model based on an asymptotic expansion of the first Born approximation and the radiation tracks were simulated with a Monte-Carlo code (Sideris et al 1993).

However, because in most practical applications with α particles of low and intermediate energies the Linear Energy Transfer is the dominant factor in considering energy deposition we developed analytical expressions for the stopping power S(E) and the range R(E) of α particles in water (Zarris, Sideris et al 1993). On the basis of these analytical expressions the LET of α particles can be calculated in the energy range of 100 keV-10 MeV which includes the energy of the α particles emitted by the ^{241}Am source of our laboratory. The mean energy deposition can also be estimated by these analytical expressions, for α particles of energies

within the same range, when the R(E) of the particle and the thickness of the water slab is known. The dependence of LET on the residual range or the residual energy of the particle emerging from the target has been also calculated (ibidem).

Energy deposition estimations, for our experimental work with aqueous DNA solutions exposed, in the form of aqueous slabs to α particles, were based on the above mentioned semi-empirical model. The methods of thermal transition spectrophotometry and perturbated angular correlations were used to study the effects of α particles vs those of gamma rays on the thermostability and the flexibility of the irradiated DNA molecules. From the thermal transition spectrophotometry measurements, the effectiveness of both types of ionizing radiation in disrupting the base pairs of the native double stranded DNA was estimated.



Relative DNA base pairs disruption per grad (K) as a function of the irradiation dose in Gy. DNA base disruption was estimated on the basis of energy propagation model

In the case of DNA molecules exposed to gamma irradiation a relatively gradual decrease of the flexibility of the molecules was observed which could be attributed to the increased presence of relatively short double stranded DNA molecules originating from double strand brakes. A somehow similar gradual effect of gamma irradiation was observed on the thermostability of the DNA molecules as well as their effectiveness on DNA base pair disruption during thermally induced helix to coil transition.

In opposite, in the case of DNA molecules exposed to α particles, the flexibility of the molecules shows a steep decrease which is associated with a sharp increase of their thermostability, above that of the non-irradiated molecules, at the range of 0-10 Gy. At higher doses of α particle irradiation the thermostability lowers down at the same level as that of the non-irradiated samples. A similar figure was

observed in the case of α particles effectiveness on DNA base pair disruption during thermally induced helix to coil transition. A sharp increase was observed at the low doses while at the higher doses the effectiveness of α particles on DNA base pair disruption lowers down to the same level as that of the non irradiated DNA molecules.

PUBLICATIONS

ZARRIS G, A ANGELOPOULOS, A PERRIS, L SAKELLIOU, E G SIDERIS (1993) **Phys. Med. Biol. 38**: 643-649

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SIDERIS E G, G ZARRIS, A ANAGNOSTOPOULOU-KONSTA, C A KALFAS (1993). DNA-water free radicals interaction and the thermostability of the DNA molecule. **Conference Proceedings SIF Vol 43**: 239-242

Head of project 6: J. Kiefer

II. Objectives:

The main objectives of the project are:
- measurements of survival, mutation induction and DNA damage after exposure to low energy protons and helium ions
- studies of single ion effects
- further development of theoretical models of energy deposition

To achieve these goals during the reporting period the following objectives were set:
- Development of irradiation facilities, both for protons and alpha-particles
- adaption of single cell techniques already available for yeast for the work with mammalian cells
- continuation of investigations on mutation induction and cell cycle progression in mammalian cells.

For the next period it is planned
- to perform inactivation and mutation experiments with low energy protons
- to study the action of single ions on mammalian cells in terms of inactivation and induction of initial DNA damage using the "comet" assay

III. Progress report:

1. Development of irradiation facilities:

There is a 2 MeV tandem generator on site which has so far mainly been used for experiments in nuclear physics. Special developments are necessary to employ this machine for the investigation of mammalian cells which were started during last year. The main problem constitutes the combination of low particle energies and the necessity to expose the biological samples in a controlled atmosphere. Exit windows have to be very thin and at the same time able to withstand about 1 bar pressure difference. A device is now being constructed which uses a small slit-window covered with 6 μm mylar foil. The cells which are deposited on membrane filters are moved over the irradiation area by means of a computer-controlled step-motor. Preliminary experiments show that this approach is feasible and that the spatial fluence distribution is sufficiently homogeneous.

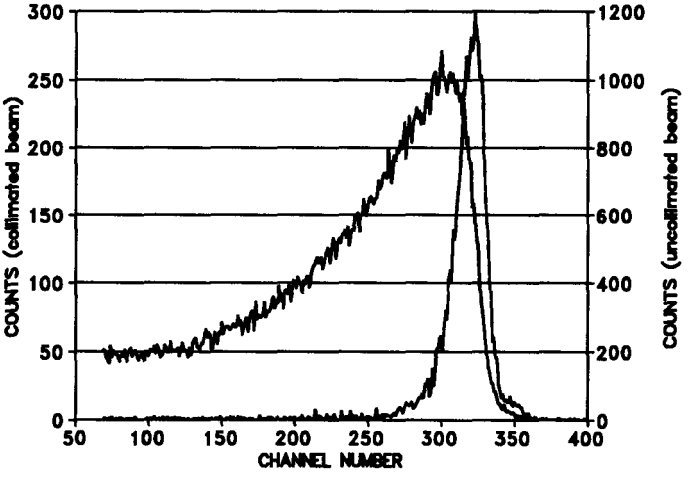

Fig 1: Energy spectra of the collimated (broken line) and uncollimated (heavy line) alpha source

This is a necessary prerequisite for a correct interpretation of results and also for single cell investigations (see below). An ^{241}Am-source is used which permits an energy of about 3.2 MeV, corresponding to a (minimum) LET of 148 keV/μm. By changing the distance between source and sample other energies and LETs can be achieved in a well-controlled way. This new piece of equipment constitutes a significant improvement over the previously used alpha exposure unit which had an opening angle of about 18 ^{0}.
Since also alpha particles shall be used for comparative investigations a new collimated source is under construction. The collimator is made of ceramics with holes of 1.1 mm and has a transmission of 75%. The angular divergence of the particles at its exit is 11^{0} thus leading to a quite narrow energy- and hence also LET-distribution (fig. 1).

2. Single cell investigations:

The action of individual accelerated heavy ions have so far only been studied in our group with yeast cells and loss of colony forming ability as endpoint. Recently also ^{241}Am-alpha-particles were employed using a collimated exposure unit which had an opening angle of about 18 ^{0} and hence a comparatively poor LET-distribution and not very good spatial resolution. Figure 2 shows the result of a typical experiment in which also the effect of preirradiation by X-rays was studied. It is seen that the inactivating power of single alpha particles depends on the distance between the cell centre and the particle traversal (the "impact parameter"). A closer analysis revealed that this is mainly due to the fact that the nucleus lies not in the centre of the cell and not to the action of "penumbra" electrons since their range is very small. The action of single particles seems to be considerably stronger than anticipated on the basis of calculated energy depositions. Similar conclusions had previously also been reached with corresponding experiments using accelerated heavy ions (Kost and Kiefer 88). It is also seen that X-rays exert a synergistic action, particularly at larger impact parameters.

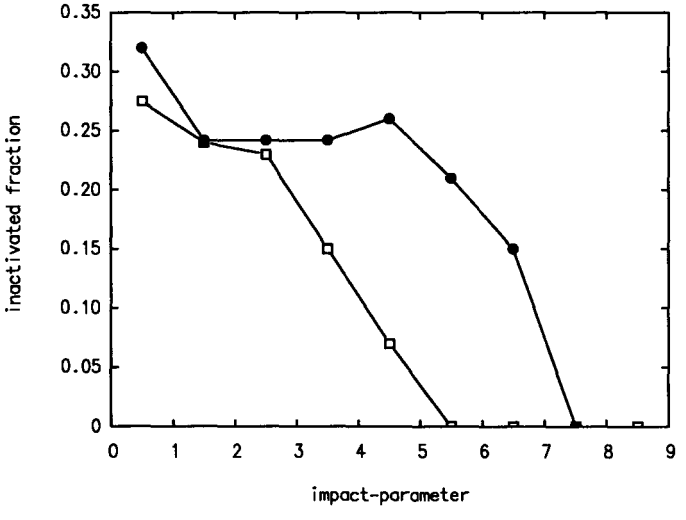

Fig. 2: Inactivated fraction of yeast cells in dependence of the impact parameter after irradiation with alpha-particles with (filled circles) and without preirradiation with X-rays (open squares);

The technique established for yeast is now being adapted to work with mammalian cells. In this case not only survival will be investigated but also initial DNA-damage by employing the so-called "comet assay" (Ostling and Johanson 84). For its quantification by computer-aided image analysis new software is being developed. Experimental preliminary studies with V79 Chinese hamster and P3 human cells are being performed in parallel.

3. Other cellular studies:

A considerable data base for mutation induction by very heavy ions in V79 Chinese hamster cells has now been established which gives a very good baseline for similar studies with human cells and lighter particles which form part of the present project. In cooperation with G. Speith and his coworkers (University Ulm) the molecular structure of mutations in the HGPRT-gene is determined. First results indicate that apart from major deletions also a high proportion of "small changes" (tentatively identified with point mutations) is found. This is a surprising result and will prompt further theoretical investigations of the microstructure of energy deposition.
Cell-cyle progression in V79 Chinese hamster cells was measured cytofluorometrically by a double-labelling technique after alpha-exposure. There is mainly a pronounced G2-delay but also a smaller retardation at the G1/S-borderline. Alpha particles are considerably more efficient than X-rays.

References:

Kost, M., Kiefer, J. (1988): Biological action of heavy ion irradiation on individual yeast cells
In: Terrestrial space radiation and its biological effects; P.D. McCormack, C.E. Swenberg, H. Bücker (eds.), Plenum Press, New York, p. 197-203

Ostling O., Johanson K., (1984): Microelectrophoretic study of radiation-induced DNA damages in individual mammalian cells.
Biochem. Biophys. Res. Comm., **123**, p. 291-298

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Kiefer, J., Stoll, U., Schneider, E. (1993): Mutation induction by heavy ions
Adv. Space Res., in the press

Kiefer, J. (1993): Problems, pitfalls, perspectives and potentials of quantitative theoretical models for cellular radiation action
Mutat. Res., in the press

Kiefer, J., Ikpeme, S., Akpa, T. C., Weber, K. J. (1993): DNA double strand break induction by very heavy ions: dependence on physical parameters
Radiat. Protect. Dosimetry, in the press

Kost, M., Kiefer, J. (1993): Biological action of single accelerated heavy ions on individual yeast cells
Adv. Space Res., in the press

Löbrich, M., Ikpeme, S., Haub, P., Weber, K. J., Kiefer, J. (1993): DNA double strand break induction in yeast by X-rays and α-particles measured by pulsed field electrophoresis
Int. J. Radiat. Biol., in the press

Progress Report

Contract: **FI3P-CT920063** **Sector: B13**

Title: New technologies in the automated detection of radiation-induced cytometric effects.

1)	Aten	Univ. Amsterdam
2)	Nüsse	GSF
3)	Bauchinger	GSF
5)	Green	MRC

I. Summary of Project Global Objectives and Achievements

When accidental exposure to ionizing radiation occurs, fast, accurate and reliable methods for the assessement of doses which human individuals or groups have recieved are needed to estimate whether intervention measures are required. Automated analysis of radiation induced chromosomal changes, once having met these requirements, could be used for a rapid screening of humans exposed to low doses of ionizing radiation.

The global objectives of this project are to develop methods for simplified and automated assessment of radiation induced damage in chromosomes and nuclei, and to make these available for radiation protection purposes.

Achievements have been made in the following fields:

- preparation and staining of chromosomes in metaphase and interphase and of micronuclei, suitable for quantitative analysis,
- automated analysis of radiation induced changes in chromosomes, interphase nuclei and micronuclei by fluorescence microscopy, by confocal scanning microscopy and by flow cytometry.
- assessment of dose-effect relationships for radiation protection purposes.

Head of project 1: Dr. Aten

II. Objectives for the reporting period

The project is directed at the development of two methods for the automated analysis of radiation effects on chromosomes.

The aim of the first part of this project was to make available the method, developed in our laboratory for the detection of dicentric chromosomes by slit-scanning flow cytometry, for application in standard cell sorter instruments. In addition, investigations are being performed to increase the senstivity of the slit-scanning analysis.

The second part of the project is directed at the analysis of the chromosomal distribution and structure in interphase nuclei. Knowledge of chromosome organization during interphase is important for understanding cellular mechanisms involved in the induction of chromosome aberrations. For these investigations, methods are being developed for the 3-dimensional analysis of dynamic processes in cell nuclei.

III. Objectives for the next reporting period

The sensitivity of the slit-scanning analysis of chromosomes will be increased by augmenting the signal-to-noise ratio in the fluorescence profiles. To achieve this we will construct a new detector, based on the method of "Time Delayed Integration", to resolve fine details in slit-scan signals that correspond to selected regions of chromosomes, highlighted by fluorescent labels.

For the analysis of chromosome organization in interphase cells we will continue the development of two and three colour confocal microscopy in combination with molecular DNA-labelling techniques. This includes 3-D image analysis techniques for the automatic analysis of changes in size, shape and position of (parts) of chromosomes in the interphase nucleus.

IV. Progress achieved including publications

Slit-scanning of chromosome aberrations

The method of slit-scanning flow cytometry developed for the detection of chromosome aberrations was modified for implementation in standard, commercially available cell sorter systems. This will make it possible for investigators in other institutes to evaluate the method and to suggest changes. For transferring the technique, the prototype of the electronic module for high-speed on-line analysis of slit-scanning signals was first tested for compatibility with other sorter instruments. A new lens system was developed to improve the optical

characteristics of standard cell sorter systems and a flow cell was constructed that can be fitted in various types of instruments.

For an optimal and reliable detection of dicentric chromosomes, all centromeres should be recognized as dips in the slit-scan chromosome profiles. Slit-scan profiles with two dips that correspond with dicentric chromosomes should be distinguished from profiles generated by scanning aggregates of chromosomes. In an earlier phase of the project we investigated which profile characteristics correspond with dicentric chromosomes, Fig.1. Among these, the depth of the centromere dips and the degree of symmetry of the profiles were effective predictors, Fig.2. During the most recent period we have worked on the construction of a new electronic module that selects symmetrical profiles with two dips. This module, moreover, is programmed to accept only those profiles that have centromeric depressions falling within a small preselected intensity range.

Sensitive immuno- and in situ- labelling techniques can be used for highlighting specific regions of chromosomes. Labelling with fluorescent markers of chromosome centromeres in particular, can be extremely useful for the detection of dicentric chromosomes. In this project, the effectiveness of techniques for fluorescent labelling of centromeres will be assessed for application to chromosomes in suspension. The advantage of these modern cyto-chemical techniques is their specificity for selected areas of chromosomes. The resulting labelling patterns show a high degree of contrast, indeed, but the intensities of the resulting fluorescence signals are too low for detection by current slit-scanning flow cytometry methods. To overcome this problem, we have designed a new type of detector with a much higher sensitivity. The detector is based on the method of Time-Delayed-Integration (TDI).

Fig. 3 shows a schematic representation of the TDI detector. During the scanning, a strongly enlarged image of the chromosome is projected on the surface of the detector. The detector contains a number of parallel segments. Each segment consists of a thin strip of optical fiber that transports the fluorescence light to a light-sensitive sensor. This sensor transforms the light signal into an electrical pulse. During the scanning, the fluorescence image of the chromosome moves across the front ends of the optical fibers mounted in the detector surface. All the detector segments "see" the complete image as a time-dependent light pulse, but there is a time shift between the pulses registered by different segments. The output signal from each segment is delayed and then added to the output signal of the next segment. The detector thus functions as a "conveyor belt" moving in synchrony with the fluorescence image that is projected on it. In this way an electrical "image" of the fluorescence profile is accumulated on the conveyor belt.

The strips of optical fibers are joined together at the front plane of the detector by a metal cylinder. The front end of the detector is placed in the image plane of the detector lens. The fibers emerging from the cylinder are connected to individual sensors, Fig. 4. The optical part of this new detector has been completed.

During the next period of the project, the detector will become operational. We will then evaluate its sensitivity and spatial resolution and its suitability for slit-scanning analysis of chromosomes.

Chromosome analysis in interphase nuclei

Although various models for the formation of chromosomal aberrations have been proposed, the mechanisms remain obscure. Little is understood of the factors that affect the way in which the initial damage caused by radiation leads to aberrations. The relative probabilities for restitution of damage and for the formation of aberrations are presumably functions of the number of damage sites produced, of the rate of repair, and of the time required for damage to promote rejoining of chromosomes in new combinations. The latter may depend on the nature and site of damage, on the distance between sites of interaction, on the way in which the chromosomes are organized in the interphase nucleus with respect to one another, and on the rate of movement of the chromatin within the nucleus.

In this part of the project we are developing methods for the quantitative investigation of the 3-dimensional organisation of the cell nucleus. We are introducing the confocal Scanning Laser Microscope (CSLM) for the 3-D imaging of fluorescence signals in the interphase nucleus. In addition we are establishing various combinations of molecular DNA-labelling techniques using replication markers and FISH. Using CSLM in combination with these labels we have started the investigation of chromosomal locations and movements in the interphase nucleus as a function of the cell cycle.

During the past period we have focussed our attention on the cytochemical aspects of the project. Important for this work is the specificity of the staining of selected chromosomal regions and the intensities of the fluorescence signals of the labelled chromosome regions. In collaboration with the Leiden and Groningen groups, we are working on methods for the production of chromosome libraries for FISH labelling of specific chromosome combinations. In these experiments, the slit-scan sorting technique is used to collect chromosomes. DNA-probes are then produced by amplification of the chromosomal DNA using the PCR technique.

Variation between cells in morphology and cell cycle phase are factors that renders the systematic study of the spatial organisation of cell nuclei highly complex. The problem of inter-cellular variation, however, can be partly ovecome by the introduction of double labelling methods. Using double labelling procedures, two fluorohromes, corresponding to two different labels, can be analyzed in the same nucleus. In this way the relative positions of selected nuclear regions can be studied in individual cells. This makes it possible, for example, to investigate dynamical processes in the nucleus as a function of the cell cycle. For this purpose we have developed a fluorescence double labelling procedure that very effectively

distinguishes between two replication markers incorporated in the cell nucleus at different periods during the cell cycle.

Publications:

Aten JA, Bakker PJM, Stap J, Boschman GA, Veenhof CHN (1992). DNA double labelling with IdUrd and CldUrd for spatial and temporal analysis of cell proliferation and DNA replication. Histochem J. 24: 251-259.

Manders EMM, Stap J, Brakenhoff GJ, van Driel R, Aten JA (1992). Dynamics of three dimensional replication patterns during the S-phase, analyzed by double labelling of DNA and confocal microscopy. J. Cell Sci. 103: 857-862.

Heilig R, Hausman M, Rens W, Aten JA, Cremer C (1993). Time optimized analysis of slit-scan profiles on a general purpose personal computer. Comp. Appl. Biosc. 9: 381-385.

Rens W, Boschman GA, Manders EMM, Slater RM, Aten JA (1993). Flow cytometric detection of chromosome abnormalities by measurement of the DNA base composition, DNA content and centromeric index. (Submitted)

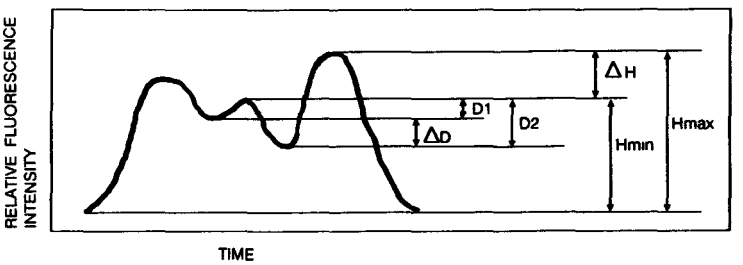

Fig. 1. Two pulse-shape parameters that were used to characterize slit-scan profiles corresponding to dicentric chromosomes. The first parameter is $\Delta H/\sum H$ with $\sum H = H_{max} - H_{min}$ and $\sum H = H_{max} + H_{min}$. The second parameter is $\Delta D/\sum D$ with $\Delta D = D_1 - D_2$ and $\sum D = D_1 + D_2$.

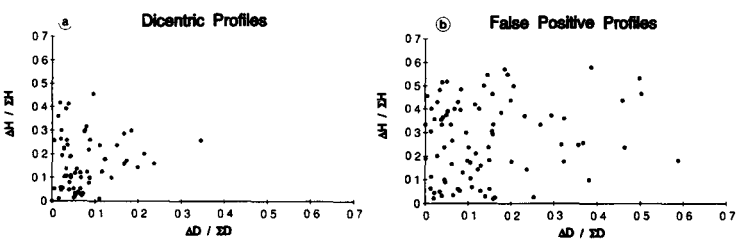

Fig. 2. (a) The values of the parameters $\Delta H/\sum H$ and $\Delta D/\sum D$ calculated for trimodal slit-scan profiles corresponding to dicentric chromosomes, plotted in a bivariate distribution. (b) The values of the parameters $\Delta H/\sum H$ and $\Delta D/\sum D$ calculated for trimodal slit-scan profiles corresponding to chromosome aggragates, plotted in a bivariate distribution.

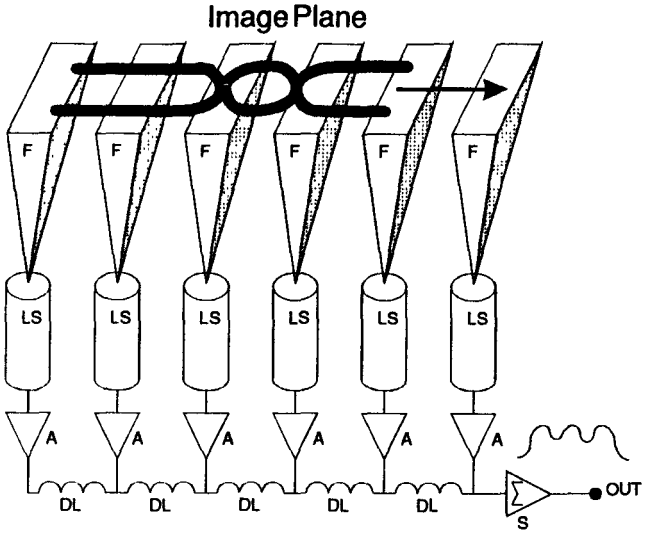

Fig. 3. Schematic representation of the "Time Delayed Intergration" detector for the sensitive detection of slit-scanning signals. A: amplifier, DL: delay line, S: summation of signals, LS: light sensor, F: optical fiber.

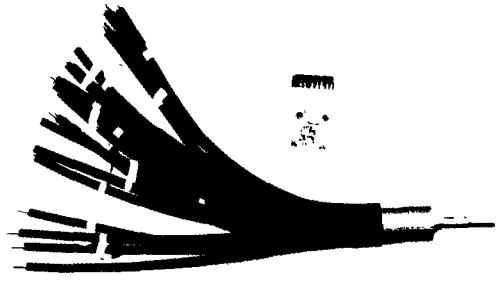

Fig. 4. System of optical fibers strips constructed for the TDI detector.

Head of project 2: Dr. Nüsse

II. Objectives for the reporting period

1. Improvement of the flow cytometric micronucleus assay using antibodies conjugated to magnetic beads that allow the separation of a specific lymphocyte population after culturing. Comparison of data obtained with the improved flow cytometric micronucleus assay with data obtained by microscopic scoring using the cytochalasin B-technique.
Development of new techniques to analyse the chromosomal composition of micronuclei using fluorescence in situ hybridization (FISH) with telomeric and centromeric DNA probes as well as chromosome painting probes on sorted micronuclei. Flow cytometric analysis of micronuclei induced in human cell lines with different radiation sensitivity.

III. Progress achieved including publications

1. Improvement of the flow cytometric technique for micronucleus scoring in human lymphocytes.

Scoring of micronuclei (MN) provides a quantitative measurement for the degree of cytogenetic damage in cells and is, therefore, increasingly used to quantify cytogenetic damage in the human population for biomonitoring studies and for the dose estimation of humans exposed to ionizing radiation. Although the conventional MN test using the cytochalasin B - technique is an established method, it cannot yet provide the capacity to screen larger human populations if more than the usual number of 1000 binucleated cells have to be analysed, especially in persons exposed to very low doses of ionizing radiation. We have therefore developed an automated flow cytometric technique for scoring MN in cultured cells and human lymphocytes (Schreiber et al., 1992 a,b). In cultured cells a good discrimination between MN and unspecific debris was possible and microscopic and flow cytometric data were usually found to agree very well. In human lymphocytes, however, this was not always the case. Comparison of flow cytometric data with microscopic measurements showed that the flow cytometric results were usually higher compared to results obtained by microscopic scoring. This effect was found to be caused by unspecific, DNA containing particles that overlap MN during flow cytometric measurement. These particles were produced by dying cells in the population of lymphocytes during cultivation in vitro necessary to express micronuclei.
An improvement of the original flow cytometric technique was therefore developed to bypass this problem. Using magnetic beads (Dynabeads, DYNAL INTERNATIONAL, Oslo, Norway) conjugated to antibodies against the CD2 antigen expressed on T-cells it was possible to select intact T-lymphocytes from cellular debris using magnetic separation (Viaggi and Nüsse, in preparation). With this improved technique lymphocytes from several donors were irradiated in vitro, cultivated for 60 h, and micronucleus frequency was measured with the flow cytometric technique in a suspension of nuclei and micronuclei prepared from magnetically separated intact T-lymphocytes. These data were compared with results obtained from microscopic scoring in the same blood samples using the cytochalasin B - technique. Fig. 1 shows dose effect curves of 7 donors measured by flow cytometry (a) and by microscopic scoring (b). Fig. 2 shows a comparison of flow cytometric results with results obtained by microscopic scoring. Fig. 1 and 2 demonstrate for the first time that the results of both techniques agree qualitatively, but also that small quantitative differences between both techniques can still be observed. The reasons for these differences are probably unspecific debris during flow cytometric measurement, but also systematic errors during microscopic scoring could cause the differences in the results shown in Fig. 2.

2. Analysis of radiation-induced micronuclei using FISH

The chromosomal composition of micronuclei induced by ionizing radiation was studied in mouse 3T3 cells for a better understanding of the DNA distribution of micronuclei (Nüsse et al., 1992). A new technique was developed to analyse the chromosomal composition of micronuclei (Miller and Nüsse, 1993). From a suspension of micronuclei and nuclei micronuclei with specific DNA content were sorted on glas slides and hybridized to a murine centromere-specific gamma-satellite DNA probe. A clear correlation between DNA content of micronuclei and number of centromere hybridization signals was obtained. The results of the in situ hybridization experiments are summarized in Fig. 3 a, Fig. 3b shows the DNA distribution of radiation-induced micronuclei measured by flow cytometry. 90% of the micronuclei with DNA content between 1 and 2% of the G1-nucleus contained no hybridization signal demonstrating their origin from acentric chromosome fragments. With increasing DNA content the frequencies of micronuclei containing 1, 2 or 3 hybridization signals increased demonstrating the presence of one or several chromosomes in micronuclei. These data agree well with results obtained in the same cells but analysed with FISH on slides using a combination of telomere and centromere DNA probes (Miller al al., 1992).

3. Micronucleus induction in human cell lines with different radiation sensitivity

Flow cytometry was used to analyse micronucleus induction in several human cell lines obtained from patients with different syndromes: aniridia (JEOLI, normal karyotype), breast cancer (TEMAR, $21q^{+}$), ataxia telangiectasia (CV56, normal karyotype) and a chromosome instability syndrome (ANDMA, abnormal chromosome 8, 11, 21). These studies were performed in cooperation with Dr. Anne Slavotinek (MRC Human Genetics Unit, Western General Hospital, Edinburgh, Scotland). Fig. 4 shows the micronucleus frequency as function of dose in these four cell lines. ANDMA was found to be the most radiation sensitive line, JEOLI the most resistant line. Similar results were obtained by microscopic scoring using the cytochalasin B - technique (performed by A. Slavotinek, not shown). Currently, the chromosomal composition of sorted micronuclei in these four cell lines is studied using several chromosome painting probes and FISH. These experiments are performed for a better understanding of the processes leading to the induction of micronuclei by ionizing radiation (random breakage or non-random breakage) in normal human cells and in cells obtained from patients with various hereditary or non-hereditary diseases.

List of publications:

Miller, B.M., Werner, T., Weier, H.-U., Nüsse, M. (1992) Analysis of radiation-induced micronuclei by fluorescence in situ hybridization (FISH) simultaneously using telomeric and centromeric DNA probes. Radiat. Res. 131, 177-185.

Miller, B.M., Nüsse, M. (1993) Analysis of micronuclei induced by 2-chlorobenzylidene malonitrile (CS) using fluorescence in situ hybridization with telomeric and centromeric DNA probes, and flow cytometry. Mutagenesis 8, 35-41.

Nüsse, M., Kramer, J., Miller, B.M. (1992) Factors influencing the DNA content of radiation-induced micronuclei. Int. J. Radiat. Res. 62, 587-602.

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Schreiber, G.A., Beisker, W., Braselmann, H., Bauchinger, M., Bögl, K.W., Nüsse, M. (1992b) Technical Report: An automated flow cytometric micronucleus assay for human lymphocytes. Int. J. Radiat. Biol. 62, 695-709.

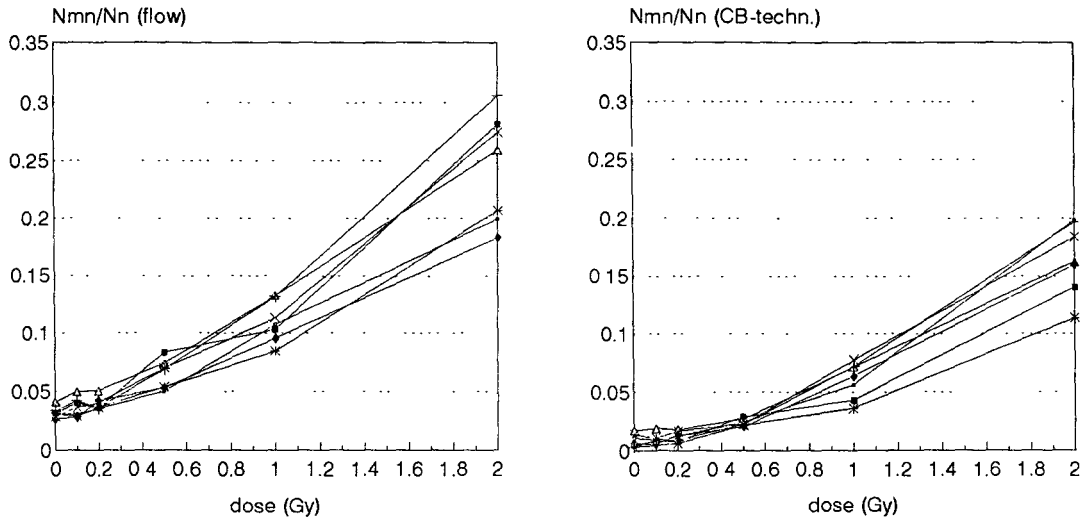


Fig. 1:
Frequency of micronuclei in human lymphocytes, N_{mn}/N_n as a function of dose measured by the new flow cytometric technique (a, left) and by microscopic scoring using the cytochalasin B - technique (b, right). The different symbols represent results obtained with lymphocytes from various donors.

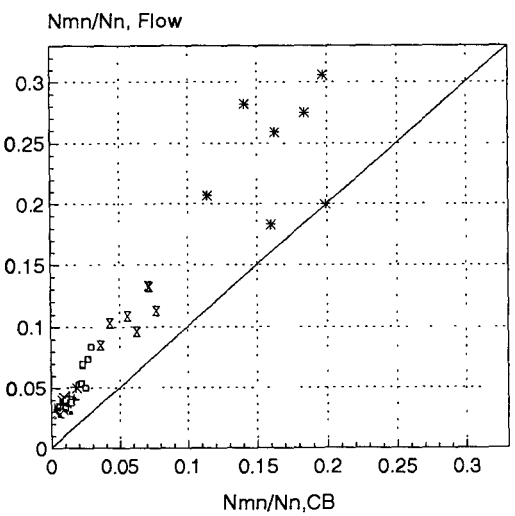


Fig. 2:
Comparison of the data shown in Fig. 1a, b. Frequency of micronuclei measured by flow cytometry, Nmn/Nn,Flow, is plotted against the frequency of micronuclei measured by microscopic scoring, Nmn/Nn,CB. The different symbols represent data from the various doses.

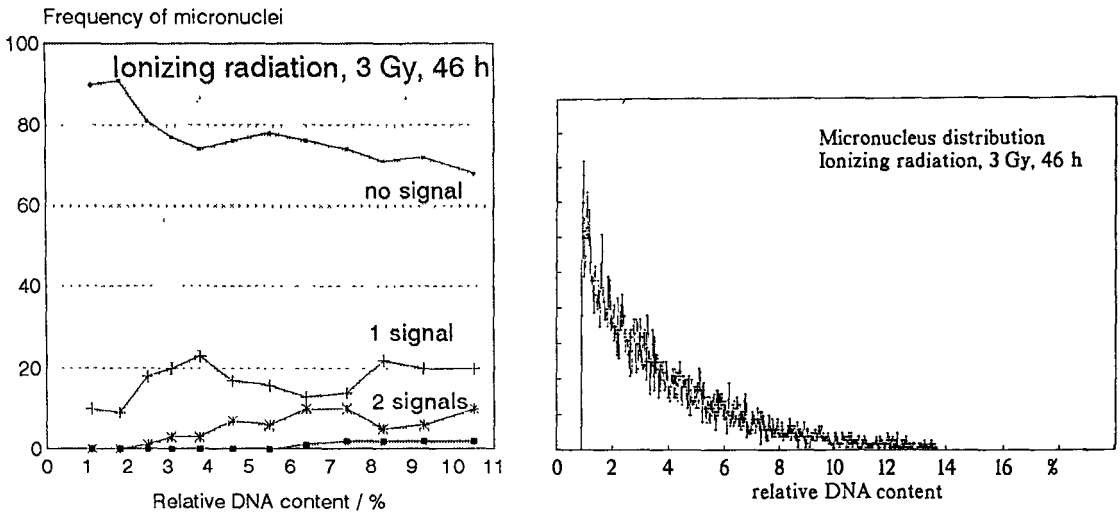


Fig. 3:
a (left): Frequency of micronuclei containing no, 1, 2, or 3 hybridization signals as a function of the DNA content of the micronuclei. Mouse NIH-3T3 cells were irradiated with 3 Gy, micronuclei were sorted according to DNA content 46 h after irradiation. Hybridization was performed with a mouse gamma satellite DNA probe.
b (right): DNA distribution of radiation-induced micronuclei measured in NIH-3T3 cells by flow cytometry.

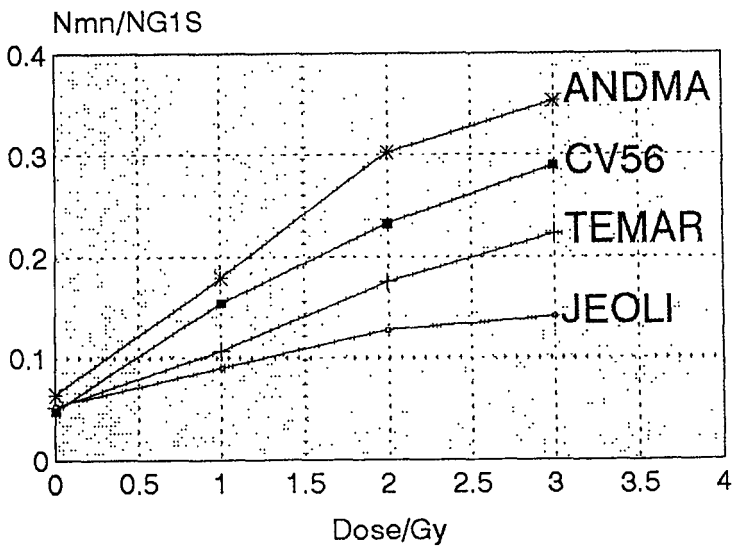


Fig. 4:
Micronucleus frequency as a function of dose in four human cell lines with different radiation sensitivity.

Prolongation of the project from June 1, 1994 until June 30, 1995

Objectives for the new project:

The main purpose of our new project is to study the processes that lead to the formation of radiation-induced micronuclei in human lymphocytes of normal donors and of donors with increased radiation sensitivity. Flow cytometry will be used to measure frequency and DNA distribution of radiation-induced micronuclei in lymphocytes of normal donors and donors with various diseases. Fluorescence in situ hybridization with chromosome specific painting probes will be used on micronuclei sorted according to DNA content to study whether random breakage or non-random, chromosome-specific breakage is responsible for the increased radiation sensitivity of these donors. The flow cytometric micronucleus assay will further be improved taking into account additional parameters for the discrimination of micronuclei from unspecific debris. New membrane specific dyes in combination with DNA specific dyes will be used for this purpose.

Progress Report

Contract: FI3P-CT92-0063 Sector: B13

Head of project: 3: Bauchinger Manfred, Institut für Strahlenbiologie, GSF

II. Objectives for the reporting period

Refinement and implementation of the technique of fluorescence in situ hybridization (FISH) for chromosome painting to measure radiation-induced translocations in human peripheral lymphocytes. Generation of dose-response curves which can be applied for dose estimations, particularly for old exposures. Application of two-colour hybridization using painting probes and a pancentromeric probe for a reliable discrimination of unstable and stable translocations. Use of a pancentromeric probe in a CB micronucleus assay with human lymphocytes to discriminate between micronuclei containing whole chromosomes or acentric fragments.

III. Progress achieved

In a first experiment with 220 kV X-rays a linear-quadratic calibration curve for stable translocations based on 17 000 cells (7 doses between 0 to 3.0 Gy) was generated. Composite whole chromosome-specific DNA probes labelled with biotin were used for a combination of chromosomes 1, 4 and 12 (DNA content about 20% of the genome). Bound biotinylated probes were detected with streptavidin FITC conjugate. Propidium iodide was used as a counterstain. Translocations could be efficiently detected by FISH. Their frequenciess were 1.8-fold higher than the frequencies for dicentircs at a given dose. The dose-response curves for translocations and dicentrics were linear quadratic with a significant higher quadratic component for translocations (Table).

The finding of an excess of translocations as compared to dicentrics started the question whether in the FISH assay misscoring of dicentrics in favour of translocations is a sufficient explanation for the observed differences. It had to be further cleared up whether scoring of stable translocations in unstable cells can influence a FISH-based quantification of translocation data.

In a 2nd experiment with ^{137}Cs γ-rays (0 to 6.0 Gy) translocations were measured by two-colour FISH with the biotin-labelled chromosome cocktail of #1, #4 and #12 (probe detection with FITC) together with a digoxigenin-labelled degenerate α-satellite pancentromeric DNA probe which was detected by sequential incubation with mouse anti-digoxigenin antibodiy, rat anti-mouse IgG and mouse anti-rat IgG both labelled with AMCA. Propidium iodide was used for counterstaining.

The background frequency of symmetrical translocations was $1.6x10^{-3}$ (11 411 cells from 11 individuals). Despite subtracting this value from induced frequencies at the various doses, similar as in the X-ray experiment, about 1.3 to 1.8-fold more translocations were found than dicentrics. Since the two-colour FISH provides a precise centromere detection in the painted chromosomes, the observation of higher translocation than dicentric frequecnies cannot be explained by misscoring.
Based on about 17 000 cells, a linear quadratic calibration curve for stable translocations was generated. Similar to dose-response data obtained from conventional scoring of dicentrics, γ-rays were less effective in producing translocations than X-rays (Table).
With increasing dose, the number of cells containing both, symmetrical and asymmetrical translocations in painted chromosomes also increases. In addition, dicentrics and acentrics are also present in the propidium iodide fluorescing part of the genome. As a consequence, a certain proportion of stable aberrations will be scored in unstable cells which have a selective disadvantage during cell proliferation. Whereas conventional dicentric scoring can be performed under cell cycle-controlled conditions, FISH analysis is not restricted exclusively to 1st division metaphases. This may influence the quantification of FISH-based translocation analyses. Using a pancentromeric probe, the analysed cells could be adequately classified into stable and unstable cells. Translocation frequencies determined only in stable cells were not found to be substantianlly different to total translocation frequencies, determined also in cells containing additional unstable chromosomal changes. Thus, scoring of translocations must not be restricted to stable cells only.

Experiments with human lymphocytes in the CB micronucleus assay were performed to apply FISH for a discrimination between micronuclei containing centromeres (i.e. chromosomes) or acentric fragments. In similar experiments as already described for mouse liver cells (see previous report) human lymphocytes were treated with the spindle poison vinblastine sulfate or irradiated with Cs γ-rays. Centromere positive micronuclei were detected with a human digoxigenin-labelled α-satellite pancentromeric probe, exclusively in binucleate CB cells stained with propidium iodide. The system could be successfully established and the chromosome preparations are completely finished. The quantification was, however, postponed in favour of the painting studies. Results will be presented in the next report.

Publications:

E. Schmid, H. Zitzelsberger, H. Braselmann, J. W. Gray, M. Bauchinger: Radiation-induced chromosome aberrations analysed by fluorescence in situ hybridization with a triple combination of composite whole chromosome-specific DNA probes. Int. J. Radiat. Biol. 62 (1992) 673-678.

M. Bauchinger, E. Schmid, H. Zitzelsberger, H Braselmann, U. Nahrstedt:: Radiation-induced chromosome aberrations analsed by two-colour fluorescence in situ hybridization with composite whole chromosome-specific DNA probes and a pancentromeric DNA probe. Int. J. Radiat. Biol. (1993) in press.

Objectives for the period of prolongation :

The FISH technique with chromosome-specific DNA libraries allows an easy and rapid detection of chromosome damage, in particular also of symmetrical translocations. In contrast to dicentrics, which are eliminated from circulation with increasing time post exposure, the incidence of symmetrical translocations was found to be fairly constant. Thus the increased potential to rapidly detect stable translocations makes this approach, also called chromosome painting, very attractive to quantify long-term and old human exposures.
A prerequisite for the application of FISH is the availability of appropriate in vitro calibration curves. Such curves have been previously generated in this project. Their practical application for dose estimation now needs to be evaluated and could be performed in the period of prolongation.
In cooperation with the Department of Dermatology, University, Munich we have already carried out chromosome analyses of a group of 10-15 subjects who had been exposed to radiation of the Chernobyl accident at the day of explosion and one day later. The group will be repeatedly examined in the hospital and is thus particularly useful to test whether a dose reconstruction of this old exposures can be achieved based on scoring of stable translocations.
Conventional dicentric analyses (cell cycle contolled, exclusively in 1st division metaphases) and FISH analyses (two-colour FISH with a triple combination of composite whole chromosome-specific DNA probes and a pancentromeric probe) will be performed with the following objectives:

-to compare both biodosimetric approaches
-to get informations on the temporal behavior of unstable and stable aberrations.
-to test a potential clonal expansion which, in case of an involvement of target chromosomes, may influence quantification
-to test the deviation of the 1:1 ratio of the stable to unstable damage observed in vitro.

It is also planned to store chromosome preparations on slides at -20° C for distribution to MRC, Edinburgh for comparative analysis with automated microsopy and FISH.

Table
Curve fitting using the linear quadratic model ($Y = c+\alpha D+\beta D^2$) for frequencies of translocations and dicentrics in chromosome 1, 4 and 12.

Radiation quality	Aberration type	$c \pm SE$ $\times 10^{-4}$	$\alpha \pm SE$ $\times 10^{-1} Gy^{-1}$	$\beta \pm SE$ $\times 10^{-2} Gy^{-2}$
^{137}Cs gamma	translocation	17.0±3.5	0.059±0.034	1.67±0.13
^{137}Cs gamma	dicentric	0.9±0.9	0.038±0.021	1.32±0.09
220 kV X-ray	translocation	22.6±5.3	0.094±0.047	2.86±0.30
220 kV X-ray	dicentric	-	0.103±0.029	1.24±0.21

Head of Project 5: Dr. Green

II. Objectives for the reporting period

The aim was to assemble the building blocks of a semi-automatic system for detecting and scoring random chromosome aberrations in metaphase cells on microscope slides prepared using a chromosome painting approach. Initially a cocktail of chromosome 1 and 2 paints were to be used for highlighting chromosome 1 and 2 DNA by *in situ* hybridisation. A metaphase would be scored abnormal if more than the expected four painted areas were detected. Each metaphase logged during the reporting period would be manually scored in order to accumulate a 'learning set' of data. Chromosome paints would be obtained from commercial sources.

Objectives for the next reporting period

In the next reporting period the performance of an automatic approach will be measured against the manual scoring data. Initially this will apply to experiments involving chromosomes 1 and 2 paint. A second group of chromosomes will also be painted in addition to chromosomes 1 and 2 in order to expand the amount of visible damage. Here a third fluorochrome will be required which is distinguishable from the DAPI and spectrum orange already in place. The complete system will be optimised to produce a semi-automatic approach attractive to the cytogenetic community.

III. Progress achieved including publications

Several major steps forward in fluorescence imaging have been made during the reporting period, while at the same time a steady accumulation of manually scored data has built up into a significant learning data set. Microscope slides were prepared from irradiated peripheral blood lymphocytes, cultured for 48 hours to give a reasonable yield of first division metaphase cells. Directly labelled painting libraries from chromosomes 1 and 2 (Imagenetics) were hybridised to metaphase preparations and a DAPI counterstain was applied to highlight the total DNA. Slides were randomly labelled so that knowledge of the radiation dose received by the cells was not conveyed to anyone involved in the manual scoring process. The following system components were established for capturing images of metaphase cells in two fluorescent colours, DAPI for showing all the chromosome DNA and spectrum orange for showing the chromosome paint. The same system enabled trained cytogenetic staff to examine and score chromosome damage.

1 An automatic metaphase finder for DAPI fluorescence, which generates a list of metaphase spread co-ordinates for a given slide. Focus was maintained automatically during the finding process by stopping the search at a pattern of predetermined co-ordinates to optimise image contrast. Manual intervention was required to load a slide, position a low power objective, establish approximate focus prior to automatic finding and to define a computer file name for that slide.

2 Automatic high resolution digitisation of DAPI and spectrum orange images for each item in the metaphase spread list for a particular slide. Here an operator positions a high power objective, oils the slide if required and establishes focus at the first item in the list. Following these preliminaries the digitisation is completely automatic and includes focussing the DAPI image prior to each digitisation.

3 Automatic analysis of digitised images using an algorithm to detect an abnormal distribution of chromosome paint with respect to the chromosome outlines established from the DAPI image. An abnormal metaphase, that is one showing

chromosome damage, was reported when more than four painted chromosome regions were detected.

4 User oriented manual scoring program allowing the operator to view the DAPI, spectrum orange and combined images on a high resolution computer screen and to record the analysis directly into a data base.

5 Automatic comparison of analyses recorded manually and automatically.

Lymphocytes from one individual were irradiated with 0, 0.5, 1.0 and 2.0 Gy and cultured for 48h. After hypotonic treatment and fixation the material was stored at -20° C. Slides were prepared from the stored material when required. On the motorised fluorescence microscope (Metasystems GMBH) metaphase cells were automatically searched for until a sufficient number were found. This often spanned more than one slide. An operator was in attendance for metaphase finding as explained above. A total search of one slide required about five minutes. High resolution digitisation on the other hand does not require attendance beyond the positioning of the first candidate metaphase. An eight slide magazine is currently used and assuming that a list of 100+ metaphase cells was generated for each slide, the potential for unattended digitisation and analysis of approximately 1000 cells is built into the system. This could for example constitute an automatic overnight operation. It was decided prior to the start of these experiments to aim for a 10% precision of each dose estimate. This meant that at least 100 damaged chromosomes must be recorded at each dose and from previous knowledge of expected dicentric counts this translates into the following numbers of metaphase cells which need to be analysed:-

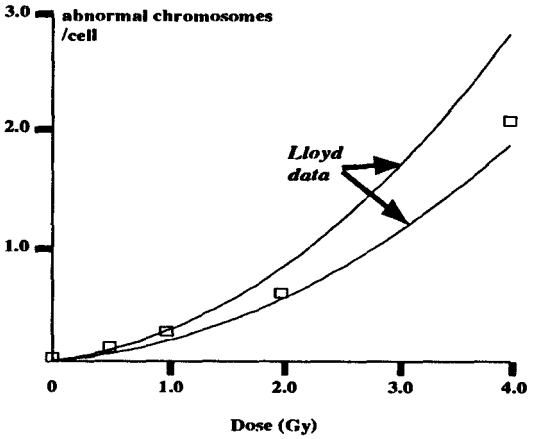
DOSE	CELLS
0.0	5000
0.5	3000
1.0	1300
2.0	425

Results.
A data base has been established of manually scored metaphase spreads. Co-ordinates, analysis (normal/abnormal/reject), detailed analysis (dicentric, translocation, which painted chromosome involved) and comments were recorded against each numbered metaphase. Accumulated numbers so far are as follows :-

DOSE	CELLS
0.0	631
0.5	416
1.0	360
2.0	239

The same numbered metaphase cells were analysed automatically, which generated a second data base where co-ordinates and analysis (normal/abnormal/reject) were recorded. The accuracy of automatic analysis, which is still in a development stage, can easily be tested whenever changes are made to the analysis algorithms.
Confidence is now established in the 'painted chromosome' approach to radiation dose measurement for peripheral blood lymphocytes following experiments in several world wide laboratories. The following graph compares our manual scoring data with data from the traditional dicentric

chromosome scoring method (Lloyd 1984).



Several assumptions were made in constructing the above graph. The Lloyd data, drawn as unbroken lines shows the upper and lower 90% confidence limits of dicentric chromosome scoring data. It has been assumed that dicentric chromosomes account for half the total amount of randomly induced damage, and therefore:-

Total **abnormal chroms./cell** = 2 x dicentric chroms./cell

Chromosomes 1 and 2 amount to 22% of the human genome and therefore lead to 33% of chromosome damage becoming visible. This statement arises from the equation:-

Visible damage = 2p(1-p) (where p = proportion of genome painted)

For the specific chromosome 1 and 2 painting approach, where an abnormal event constitutes the deletion of paint from a normally painted chromosome 1 or 2 coupled with the addition of paint to another chromosome:-

Total **abnormal chroms./cell** = 3 x abnormal events/cell

On this basis the comparison of traditional and painting results are well within confidence limits.

A fraction of the accumulated manually scored data has been analysed in greater detail, where a distinction was made between a damaged chromosome which was dicentric and one which was a balanced translocation. The following table shows the results :-

Dose (Gy)	No. cells analysed	No. dicentrics	No. translocations
0.5	414	5	9
1.0	80	1	2
2.0	157	10	24

It is clear that the ratio of translocations to dicentrics, which averages about 2:1 does not agree with previous assumptions of an equal proportion of each damage type. Further analyses will give a more precise answer and more importantly even more detailed analysis will establish whether any bias in the analytical process favours translocations.

References

Lloyd DC. (1984) An overview of radiation dosimetry by conventional cytogenetic methods. in Biological Dosimetry (Ed. Eisert WC and Mendelsohn ML) Springer, Berlin, 3-14.

Progress Report

Contract: **F13P-CT920064i** **Sector: B13**

Title: The induction of chromosomal changes in human and rodent cells by accelerated charged particles: early and late effects

1)	Edwards	NRPB
2)	Natarajan	Univ. Leiden
3)	Bimbot	CNRS
4)	Dutrillaux	CEA - FAR
5)	Kraft	GSI

I. Summary of Project Global Objectives and Achievements

The induction of chromosomal aberrations is used as one of the basic data bases for judgements made in radiological protection. It contributes to the judgements of radiation quality and to the extrapolation of risk to low doses and low dose rates. Chromosomal damage is believed to be an early step in the long sequence of events which subsequently lead to neoplastic transformation of cells. This contract aims to provide more detailed information on the radiation quality dependence of aberration production and a better understanding of the production of chromosomal aberrations both in the first cell cycle and after many cell divisions.

The first two projects use human lymphocytes irradiated in G_0 to measure dicentric yield at the first metaphase and to follow the time course of initial break repair. Using 1000 MeV oxygen-16 ions with an LET of 67 keV.μm^{-1} the dicentric yield was a factor of 3 lower than expected on the basis of LET. The reason for this possibly lies in the track structure of these ions. The initial breaks appear linear with dose with an RBE with respect to x-rays in the region of 1.3. The breaks repair marginally more slowly than those following x-irradiation.

The third project essentially controls the beam production and dosimetry. The lessons learnt so far are that fluence measurement using track etch plastic devices are still the most accurate methods of dosimetry. Their disadvantages are that they can only be used at very low total doses which involves low beam monitor currents and at present are not capable of immediate read out. The ionisation chamber operates at the dose rates used to irradiate cells, hence no extrapolation is required and gives an immediate reading. The disadvantage is that the operating characteristics need to be fully understood when using high LET particles. Columnar recombination is clearly an important phenomenon which affects ion collection.

The fourth and fifth projects concern cell instability, that is the observation of chromosomal aberrations many cell cycles following irradiation. In project 04 the cell line is the human fibroblast and the colonies have been cultured to death. There is some evidence that irradiated cells are able to undergo more passages than unirradiated cells. Further irradiations have been done using three different donors to investigate whether the predominant involvement of chromosomes 1, 13 and 16 is a specific or a general effect. In project 05, the failure to find the instability effect with CHO cells is interesting and further experiments are being carried out.

Head of project 1: Dr. Edwards

II. Objectives for the reporting period

The primary objective for this reporting period has been to score chromosomal aberrations in metaphase spreads of human lymphocytes following irradiation of blood with oxygen ions of about 63 MeV/a.m.u (1000 MeV) and then analyse the data. The irradiations took place at GANIL in the autumn of 1992 and scoring and making final estimates of dose has been a continuous process since then. A second objective has been to arrange the next visit to GANIL to irradiate with another particle and energy. This visit will include work described under projects 02 from Leiden (Natarajan) and 03 from Paris (Bimbot).

The proposed work for the next reporting period is to attend GANIL in October to irradiate blood with carbon ions of energy in the region of 95 MeV/a.m.u (1140 MeV). Blood will be cultured at GANIL and microscope slides prepared. These will be taken back to NRPB for scoring and analysis. The analysis will include results obtained from Leiden during the same visit concerning the time course of conversion of double strand breaks to dicentric chromosomes, see project 02. Attempts to arrange a subsequent visit to GANIL to use a different ion-energy combination will be made. It is also hoped during this period to arrange a joint contractors meeting with contractants in the group co-ordinated by Moschini in order to discuss results obtained within the two contracts.

III. Progress achieved including publications

Previous work in this laboratory has aimed at measuring dose effect relationships for the induction of chromosomal aberrations in human lymphocytes by radiations of many different qualities. In particular the dicentric aberration has received greatest attention because it is relatively easy and reliable to score and it has a low control level. Analysis of dose effect curves has consistently demonstrated that the yield curve

$$Y = C + \alpha D + \beta D^2$$

fits the observed data well. For research aimed at radiation protection, it has been the variation of the coefficient α with radiation quality which has received greatest attention. Previous work from just 5 neutron energy spectra from a fission spectrum to 14 MeV neutrons produced a hypothesis concerning the variation of α with LET. This hypothesised curve is shown in Fig. 1. The objective of using charged particles of nearly constant LET to irradiate blood has been to verify or revise this curve. Data for natural α-particles (120 keV/µm), 23 MeV helium-3 ions (22 keV/µm) and 190 MeV neon-20 ions (460 keV/µm) have all fallen close to this curve. Data for 9 MeV protons (5 keV/µm) lay about a factor 2 below the curve. Data for 1000 MeV oxygen-16 ions at 67 keV/µm, which have been analysed during the period of this report, showed the expected linear relationship with dose but the value for α was a factor 3 lower than predicted by Fig. 1. At least part of the reason for this low value lies in the δ-ray structure of the oxygen track. An energy as

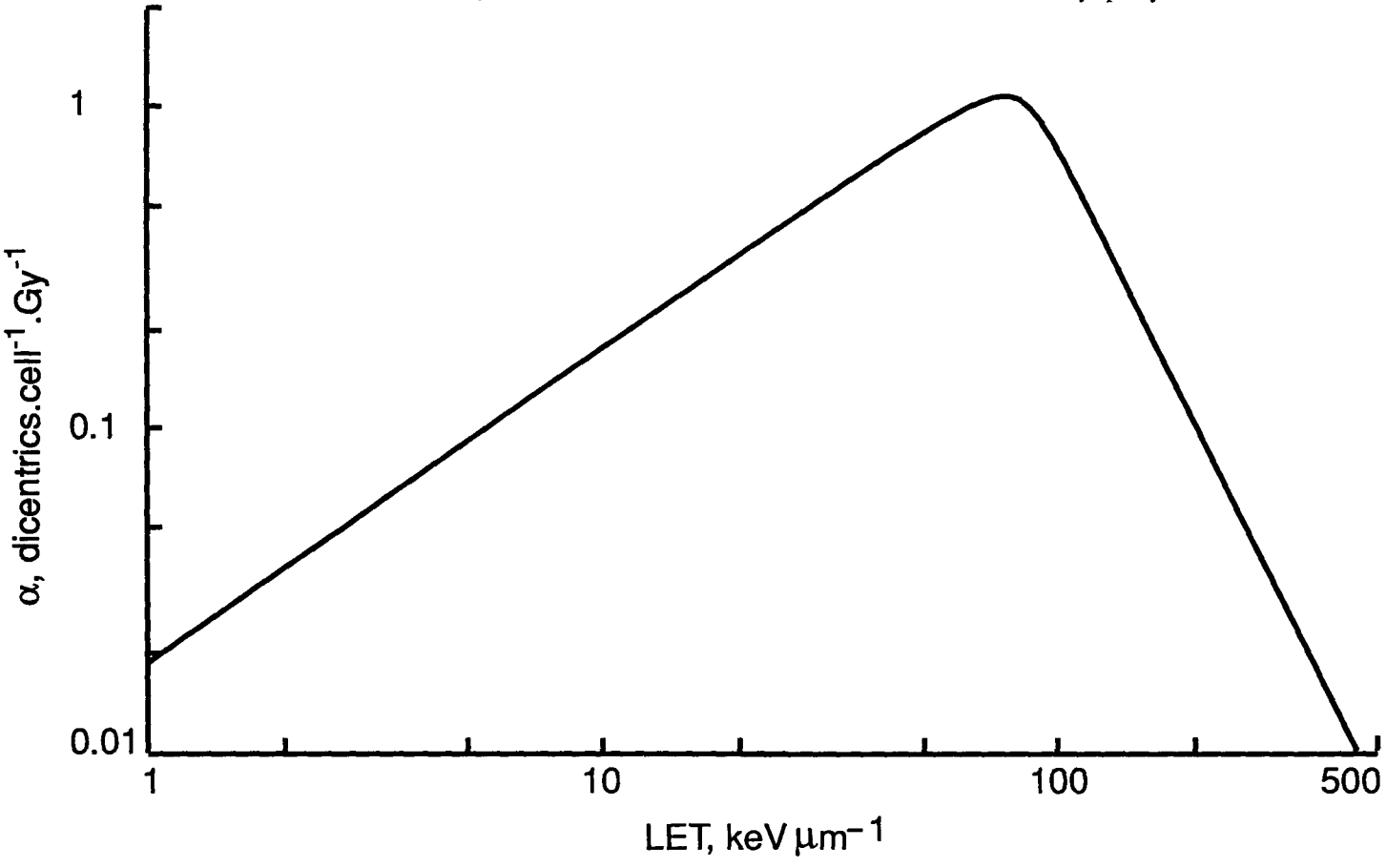
high as 63 MeV/a.m.u can produce electrons of 120 keV which have a range in tissue approaching 200 µm. This means that a particle track crossing a cell nucleus will deposit some energy well away from the track centre and hence outside the cell nucleus. The effectiveness of these high energy oxygen ions would then be more typical of a particle of lower LET_∞. Thus qualitatively these results support models based on the concepts of restricted LET and limited interaction distances. Their quantitative agreement depends upon precise details of the models and the energy deposition structure of the oxygen track.

The next visit to GANIL has been arranged for October 1993 when it is hoped that a carbon ion beam of 95 MeV/u (1150 MeV) with an LET of about 30 keV/µm will be used. Arrangements have also been made for workers from projects 02 and 03 to attend to perform their experiments.

Publication

Edwards, AA, Finnon, P, Moquet, JE, Lloyd, DC, Darroudi, F and Natarajan, AT. The Effectiveness of High Energy Neon Ions in Producing Chromosomal Aberrations in Human Lymphocytes. Radiation Protection Dosimetry (1993). To be published.

Fig. 1. A suggested variation of α with LET for dicentrics induced in human lymphocytes



Head of project 2: Prof. Natarajan

II. Objectives for the reporting period

The aim of the project was to use the accelerator at GANIL (Caen) to irradiate specimens of human peripheral blood lymphocytes with ions of nearly constant LET (in the range of 10-200 keV/μm) (ie. oxygen-16 ions of about 63 MeV/A corresponding to a LET value of 67 keV/μm) and investigate the induction of chromosomal damage and repair after irradiation using the technique of Premature Chromosome Condensation (PCC).

III. Progress achieved including publications

Human lymphocytes were isolated using LeucoPREP™ brand, cell separation tube (Becton Dickinson Labware). The resulting mononuclear cells were irradiated with oxygen-16 ions with the LET in the region of 67 keV/μm. (The dosimetric aspects are reported under project 03 of this contract). The lymphocytes were injected into sample holders (about 1 x 10^6 cells/sample holder). For each dose point 4-5 sample holders were irradiated simultaneously. Following irradiation a) lymphocytes were pooled and fused immediately, b) allowed to recover at 37°C for two hours then fused with mitotic Chinese hamster ovary (CHO) cells. The mitotic CHO cells were obtained by mitotic shake off and were prelabelled with 5-Bromodeoxyuridine (BrdUrd) for at least 2 cell cycles. Therefore, following Giemsa staining mitotic CHO cells can be differentiated from human interphase cells based on the Giemsa staining property of BrdUrd substituted chromosomes.

The lymphocytes were irradiated with doses of 0.74, 1.47 and 2.22 Gy oxygen-16 beam. A set of lymphocyte samples irradiated with a dose of 2.22 Gy was allowed to recover for two hours before fusion. Following fusion of human lymphocytes and mitotic CHO cells in the presence of polyethylene glycol (MW = 1450), cells were allowed to grow for one hour in complete growth medium in the presence of Colcemid. Routine air-dried preparations were made and slides were stained according to the fluorescence plus Giemsa technique. 40-52 cells were scored for each dose point. The frequency of PCCs in human lymphocytes irradiated with the oxygen-16 beam are presented in Table 1 and Fig. 1. The data for kinetics of repair of

lymphocytes after irradiation with oxygen-16 ions are presented in Fig. 2.

The number of elements in each metaphase was scored and the excess of PCC fragments above control (46 elements) was determined. The distribution of PCC fragments following irradiation with different doses of oxygen-16 ions (Table 1) indicated that lymphocytes were uniformly irradiated. Therefore data are presented as frequency of breaks per cell for different doses of oxygen-16 ions. A linear dose response curve was observed (Table 1, Fig. 1). From the PCC data the RBE at zero time with respect to x-rays is estimated to be in the range of 1.2-1.3. Following 2 hours recovery time, the frequency of breaks decreased by about 30% (Table 1, Fig. 2). The rate of recovery following oxygen-16 ions is about 20% less than the values obtained with x-rays. The implication is that breaks caused by high LET radiation repair a little more slowly than those caused by low LET radiation.

Table 1

Induction and repair kinetics of PCCs in human peripheral blood lymphocytes following irradiation with ^{16}O beam of 63 MeV/A

Dose (Gy)	Fusion time (h)	Cells scored	Distribution of PCCs															Breaks/ cells
			46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	
0	0	50	50	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
0.74	0	52	6	11	11	8	7	3	5	1	0	0	0	0	0	0	0	2.6
1.47	0	40	0	0	1	4	11	5	5	7	2	2	1	1	1	0	0	5.7
2.22	0	40	0	0	0	0	2	4	5	4	6	9	2	3	1	2	2	8.4
2.22	2	45	0	0	0	5	8	7	7	7	5	6	0	0	0	0	0	5.9

Fig. 1.

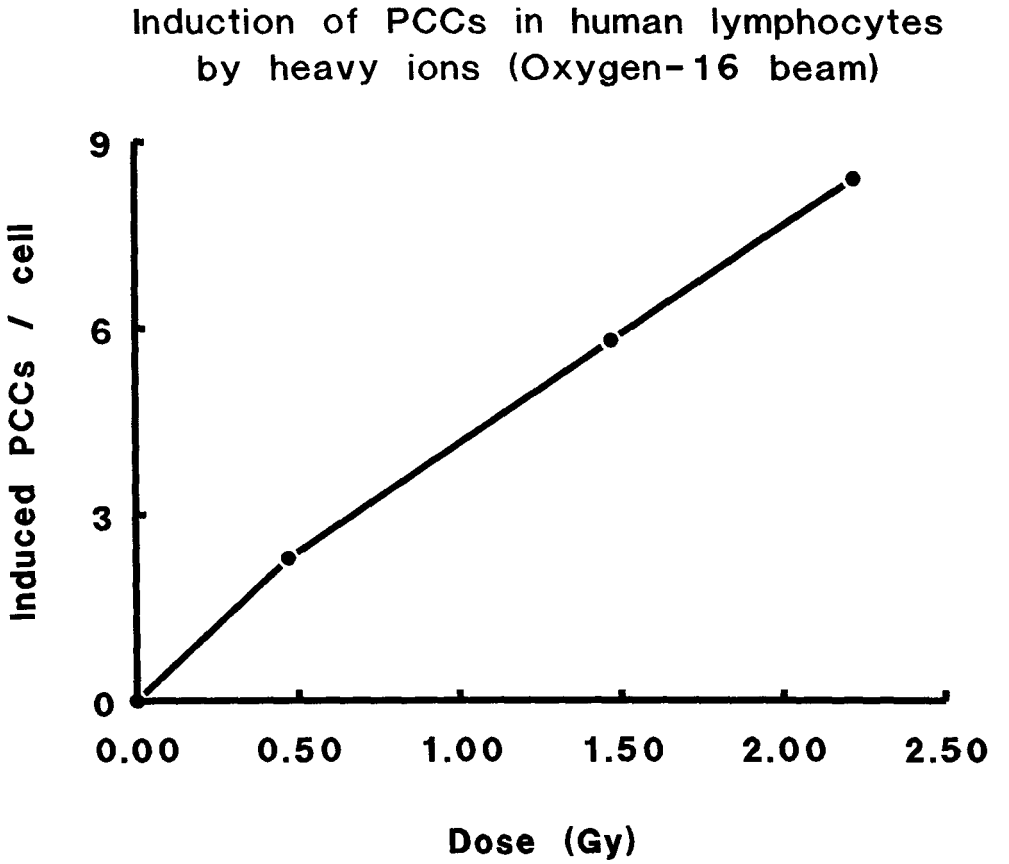
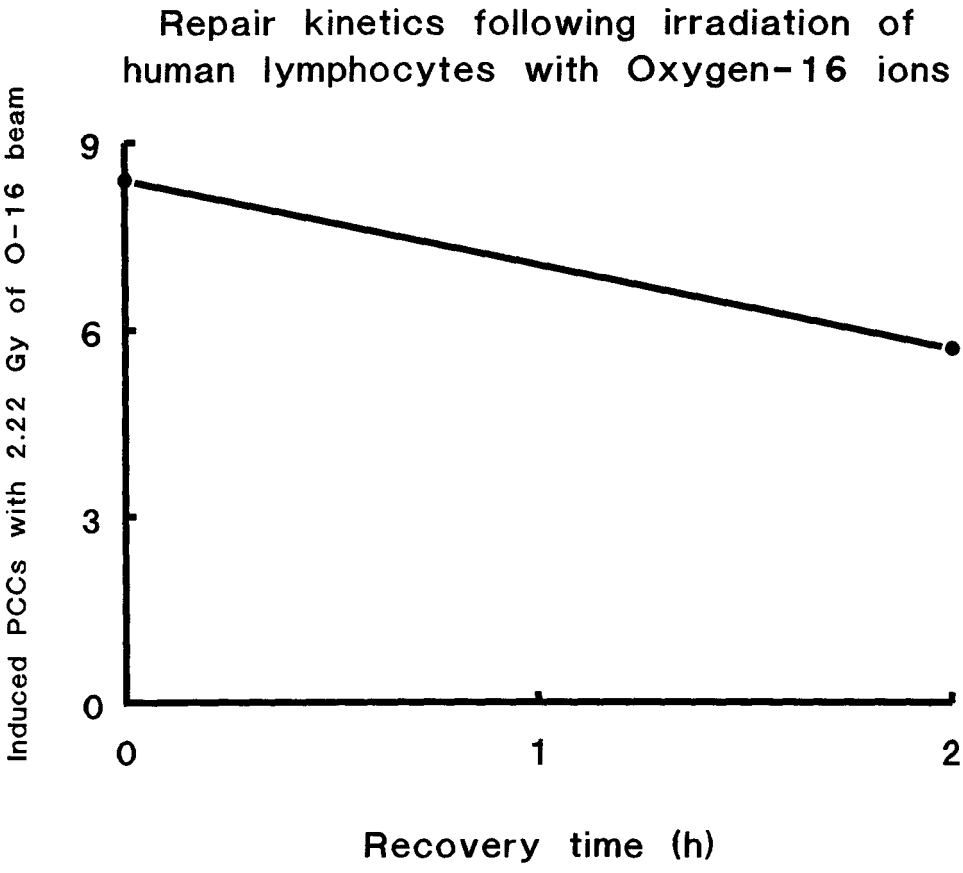

Induction of PCCs in human lymphocytes
by heavy ions (Oxygen-16 beam)
Induced PCCs / cell
9
6
3
0
0.00
0.50
1.00
1.50
2.00
2.50
Dose (Gy)

Fig. 2.


Repair kinetics following irradiation of human lymphocytes with Oxygen-16 ions
Induced PCCs with 2.22 Gy of O-16 beam
9
6
3
0
0
1
2
Recovery time (h)

Head of project 3: Dr. Bimbot

II. Objectives for the reporting period

The contribution of the IN2P3-GANIL part of the collaboration with the work of projects 01 and 02 of this contract consists of providing the heavy ion beam of proper energy and intensity, in setting up the accelerator beam line and beam delivery systems in order to deliver a light beam of sufficient homogeneity to satisfy the requirements of the biology experiment, and in measuring the beam fluence with the best possible accuracy, the applied dose being determined from this fluence measurement.

For the reporting period, in which this work was initiated, the objectives were set up and check all the concerned apparatus, and to realise a first biology experiment.

For the next reporting period, the objectives are the following:

i) improve the performances and accuracy in delivering homogenous beams, measuring their homogeneity, and the doses delivered

ii) improve the equipment of the laboratory which is used for the cell preparation and culture parts of these experiments

iii) realise two other experiments.

III. Progress achieved including publications

The work devoted to this program includes three types of contributions which differ in nature and by their degree of specificity to be adapted to the experimental purposes. These contributions are the following:

i) preparation and test of specific beam line equipment

ii) standard operation of the GANIL accelerators in order to get the proper beam at the proper time

iii) realisation of the experiment.

1. <u>Preparation and test of specific beam line equipment</u>

The realisation of the experiment requires a homogeneous beam distribution over the area of the cell containers (3 cm in diameter) and to measure accurately the dose.

As far as beam distribution is concerned, two methods can be used. The active method consists in sweeping horizontally and vertically a beam spot of weak dimensions with a couple of magnets, the fields of which are varied according to the required excursion in each direction. The passive method consists in inserting a metallic foil in order to spread the beam by "natural"

scattering as it goes through this degrader. Taking into account the distance between the scattering foil and the samples to be irradiated, the thickness of this foil has to be adjusted in order to lead to a sufficient beam homogeneity without losing too much energy or intensity. Both the active and passive methods were tried during test runs using auxiliary GANIL beams (ie. runs in which the beam is used 10% of the time for the test and 90% of the time for another experiment). During these tests, the beam homogeneity was measured using standard GANIL beam profilers which are secondary emission wire counters.

For the sake of simplicity, and because it was better adapted to the experimental set up supplied by the NRPB team, the passive method was adopted for the experiment.

In order to achieve precise measurements of the fluence, and therefore of the dose, an ionisation chamber adapted to heavy ion beams was developed at GANIL. This chamber is a very flat cylinder (3cm in diameter and 3.2mm in thickness), with thin entrance and exit windows (5 µm Ni) operating with a 70% Ar-30% CO_2 mixture. During test runs, it was checked against two calibrated ionisation chambers used in two French anticancer centres for measuring doses from gamma rays and proton beams respectively, and these checks indicated good agreement within an uncertainty of about 15%.

2. Standard operation of the GANIL accelerator

For realising the experiment in which blood samples were irradiated, but also for the test runs that have been mentioned, a heavy ion beam had to be prepared and delivered. For the test runs, two days of auxiliary beam (10% of the time) were necessary. For the preparation and realisation of the experiment, three days were necessary, half of them being used for tuning the accelerator and half for the experiment itself, including the last physical tests for beam homogeneity and dose measurements.

3. Realisation of the experiment

We will mainly present here the aspects of the experiment linked to beam delivery and intensity measurements.

The experiment was performed in the target room G4 at GANIL. In addition to the technical staff of GANIL who operated the accelerator and drove the beam to the irradiation room, three physicists from IN2P3 participated in the experiment. The aim of this first experiment was to check the operation of the whole set of apparatus, and to realise a first series of blood cell irradiations.

It was also the first opportunity to compare the beam measurements performed with the NRPB devices which had already been used to those from the newly built GANIL instruments. The NRPB team had brought a rotating wheel which was used as a sample holder in which up to 19 samples could be irradiated at a time. NRPB had also provided the experiment with a secondary electron monitor, an ionisation chamber, and a set of plastic track detectors to calibrate the beam. The GANIL ionisation chamber, and a gas profile monitor built at GANIL were also used. The gas profile monitor is a wire detector derived from the Charpak counters in which two planes of parallel wires (horizontal and vertical) are operating in gas. According to the applied voltage, this counter works in the proportional or Geiger mode, and this makes it possible to measure the beam horizontal and vertical profiles even at very low intensities (down to 10^4 ions/s).

It was originally planned to irradiate the blood samples with a ^{12}C beam of 40 MeV/A corresponding to an LET value of about 50 keV/micron, but, due to difficulties encountered in the machine tuning up, an ^{16}O beam of 65.77 MeV/A, leading to an LET of 70 keV/micron was finally delivered and accepted by the research team. The beam energy was measured by the technical

staff of the machine, using a magnetic spectrometer placed at the entrance of the experimental area.

The beam was spread out and made roughly uniform over the 3 cm diameter of the samples to be irradiated by inserting a 52 micron tantalum foil into the beam, 6m upstream from the irradiation point. The gas profile monitor indicated that the beam intensity at the centre of the irradiated zone was 20% higher than at its edges. The dose was calibrated using the NRPB ionisation chamber which operated with air, and which was placed behind a 2 cm diameter collimator at the exit point of the line. The charge deposited in this ionisation chamber (directly used to calculate the dose) was measured versus the integrated intensity delivered by a secondary electron monitor placed slightly upstream. For the irradiations, the ionisation chamber was replaced by the blood samples, and the secondary electron monitor was used to define the beam fluence to apply, according to the previous calibration.

The signals from the NRPB and the GANIL ionisation chambers were compared over a broad domain of beam intensities. These responses were also compared, at very low intensity, with the fluences from plastic track detectors. This study, the analysis of which has still not been completed, indicate a fair agreement between the measurements. However, a discrepancy is observed between the two ionisation chambers at the highest intensities explored. This could come from ion recombination in the gas of the chambers, an effect which should appear first for the NRPB ionisation chamber which is thicker by a factor 4. Studies which should lead to improvements in this domain are in progress. Comparisons of the dose measured by the NRPB chamber and the fluence measured by track etch plastic detectors showed a 40% discrepancy. This was eventually traced to incomplete ion collection in the ionisation chamber and so dosimetry was based on the plastic track etch device. The implications for dose measurement using ionisation chambers has not been fully analysed but will form the basis of further experiments.

Head of Project 4: Dr. Dutrillaux

II. Objectives for the reporting period

The project aims at studying the specific chromosomal instability detected in human fibroblasts after heavy ion irradiations.

For the reporting period three objectives were pursued.

1) To carry on the cultures of 7 irradiated cultures of the same donor, up to senescence or immortalization.

2) To perform new experiments on 3 additional donors.

3) To set up the methods to identify the type of chromosomal instability (telomeric associations or dicentrics).

The objectives for the next reporting period are:

1) To search and follow up chromosome instability and formation of clonal rearrangements by cytogenetic study, using R-banding, every five passages.

2) Development of in situ hybridization of telomere specific probes to search for the existence of telomeric sequences on unstable chromosomes.

3) Evaluation of the mean length of telomeric sequences before and after the appearance of chromosomal instability by Southern blotting.

III. Progress achieved including publications:

Specific chromosome instability detected in human fibroblasts after heavy ion irradiation

The study of the 7 cultures irradiated at GSI by Neon ions (E=10.74 MeV/u, LET=386 keV/µm, fluences of 10^6, 2.10^6 and 4.10^6 particles/cm^2), Argon ions (E=10.52 MeV/u, LET=1207 keV/µm, fluences of 10^6, 2.10^6 and 4.10^6 particles/cm^2) and Lead ions (E=9.5 MeV/u, LET=13600 keV/µm, fluence of 2.10^6 particles/cm^2) shows identical results and permits to propose the following scheme. Irradiation by heavy ions induce multiple chromosomal alterations proportional to fluence of the particles. Many cells directly affected by the track of one (or more) particles either die or do not give rise to viable descendants (majority of normal karyotypes in metaphases around the 5 - 10th passages). A transmissible chromosome instability is observed in descendant cells. This instability affects preferentially particular telomeric regions of chromosome arms 1p, 13p, 13q, 16p and 16q. Some clones, characterized by chromosome rearrangements and imbalances, progressively develop and invade all the cultures around passage 25 after irradiation (Sabatier et al, 1992; Martins et al, 1993).

Our three objectives for the reporting period have been developed as follows:

1) To carry on the cultures of the 7 irradiated cultures of the same donor, up to senescence or immortalization.

While the cell cultures were continued the lifespan of the irradiated cells was increased. Control cultures have been prolonged up to 45 passages before death whereas the irradiated cells described before have died at 47, 54, 53, 49, 56, 45 and 50 passages for the various conditions described above, respectively.

The karyotypes were performed each 5 passages up to the end of the cultures. The analysis is in progress.

2) To perform new experiments on 3 additional donors.

Our previous experiments used a single donor and the specificity of the chromosome instability detected in his fibroblasts could be a characteristic of the three chromosomes involved, or vary from donor to donor.

Four additional experiments have been performed at GSI with Gold 9.4 and 800 MeV/u, Nickel 400 MeV/u and Oxygen 11.2 MeV/u. Primary cultures of human fibroblasts from three new donors have been irradiated. The cell cultures were carried on and karyotypes established each five passages after irradiation. The work is in progress.

3) To set up methods for identifying the type of chromosomal instability (telomeric associations or dicentrics).

A specific involvement of telomeric regions may correspond to lesions of telomeric repeats, in particular deletions, as described in senescent and transformed cells. The same observations were made with ions of increasing fluences and LET. We wish to determine whether telomeres are shortened when the chromosomal instability appears. Two approaches have been developed. One is quantitative (analyses of the mean length of telomeric sequences by Southern blotting) and the other is qualitative (fluorescence in situ hybridization). Our preliminary results show no shortening of the mean length of any of the telomeres. We are now developing in situ hybridizations to look for a possible alteration of the telomeres of the chromosomes specifically involved in instability.

The characterisation of the type of chromosome instability is focussed on the distinction between telomeric associations (telomeric sequences are involved in the rearrangement) or dicentrics (telomeric sequences have been lost). Thus we need methods permitting the detection of telomeric sequences (Moyzis et al, 1989). We have adapted the methods of telomeric in situ hybridization to our needs and have successfully stained around 95% of chromosomes on both chromatids per cell (figure 1). The work is in progress with irradiated fibroblasts.

Publications

Martins B, Sabatier L, Ricoul M, Pinton A and Dutrillaux B. Specific chromosomal instability induced by heavy ions: a step towards transformation of human fibroblasts? Mutation Research 285, 229-237, 1993.

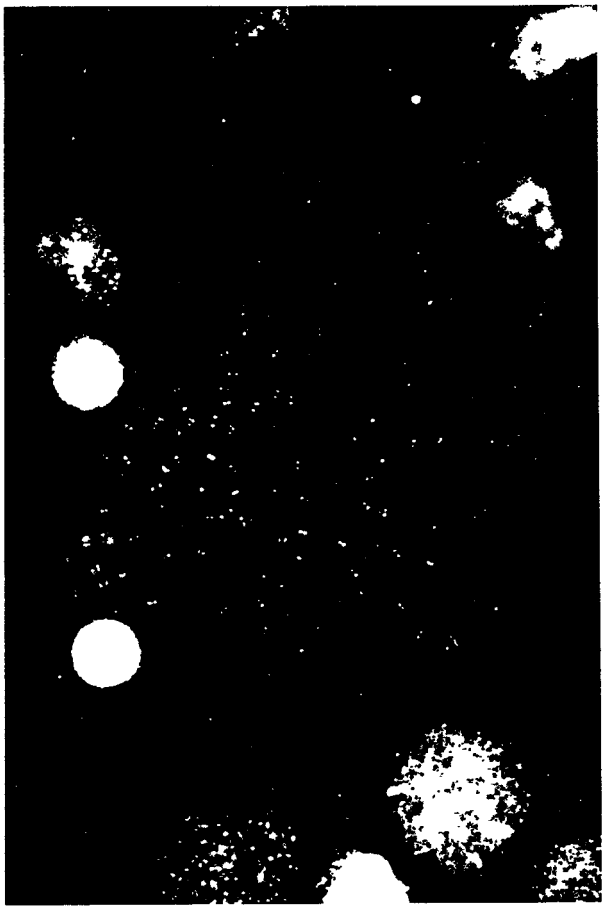


Fig. 1. Metaphase spread of a human fibroblast showing telomeric sequences

Head of project 5: Dr. Kraft

II. Objectives for the reporting period

It was proposed to start with a search of chromosomal instability in CHO Chinese hamster cells using one light ion beam at various energies and to compare the results to parallel measurements of the Paris group. Secondly, the in situ hybridization technique for the study of translocations in human hamster hybrid cells should be installed and used in the first exploratory experiments.

III. Progress achieved including publications

Experiments using an oxygen beam at two different energies did not yield evidence for chromosomal instability in CHO cells after many cell generations. Therefore, the experiment will be repeated with a different experimental protocol and extended to other ions.

The technique of in situ hybridization has been acquired and first experiments have been carried out.

Publications

S. Ritter, E. Nasonova, E. Kehr, W. Kraft-Weyrather and G. Kraft. Induction of chromosomal damage in mitotic V79 cells by heavy particles, GSI Report 93-1, 324.

E. Nasonova, S. Ritter, W. Kraft-Weyrather and G. Kraft. Induction of chromosomal damage in Chinese hamster cells by high energy heavy ion beams, GSI Report 93-1, 325.

Progress Report

Contract: FI3P-CT930067 Sector: B13

Title: Development and investigation of systems for the quantification of radiation induced carcinogenesis in humans.

1)	Mothersill	Inst. of Technology - Dublin
2)	Riches	Univ. St. Andrews
5)	Luccioni	CEA - FAR
6)	Martin	ANS
7)	Arrand	Hosp. Mount Vernon

I. Summary of Project Global Objectives and Achievements

Objectives

The main objective of the project is to define suitable epithelial and glial culture systems which will allow the investigation of the cancer risk to humans from exposure to low doses and dose rates of ionising radiations. The objective is pursued by:

1. identifying and establishing suitable human cell lines for radiation-induced transformation studies;

2. continuing animal experiments using pregnant rats, where high levels of central nervous system tumours in adult offspring can be obtained;

3. identifying suitable end-points for quantitation of transformation and for elucidating the steps in the process, using molecular biological approaches to define transformation markers and growth factor expression;

4. linking end-points defined using in vitro clonogenic assays, in vivo xenograft growth and markers.

Achievements

1. Several human cell lines and primary culture systems have now been established in the five laboratories. These range from three-dimensional tissue equivalent cultures to immortalised virus transfected cell lines and allow the radiation response of cells in various pre-neoplastic states to be examined;

2. Using three different virus immortalised cell lines it has been possible to induce tumours in nude mice. The most promising line for radiation studies appears to be the HTori thyroid line. The HPV-G line gives tumour-like growths in nude mice on injection of control cells and is not being used except for radiation survival studies;

3. p53 protein expression has been induced in the HaCaT cell line and in normal urothelium by irradiation. The methodology has been established to examine the p53 gene in these cultures;

4. Cell survival following irradiation has been accurately measured in HPV-G, SV-HUC-1 and L132 cell lines and permit quantification of transformation frequencies. Other cell lines are in the process of being quantified;

5. The rat fetal glial cell model has been developed and p53 induction is being studied with the aim of providing an in vitro/in vivo bridging system for the project.

Head of project 1: Dr. Mothersill

II. Objectives for the reporting period

1. To culture normal epithelial cells from normal and cancer-bearing individuals and following irradiation, to identify and quantify levels of expression of p53 EGF receptor and cmyc proteins.

2. To relate extent of cell growth ("hyperplasia in vitro") post-irradiation to expression of the proteins in 1 above.

3. To accumulate samples of cultures showing induction of stable (conformationally inactive) p53 post-irradiation for sequence analysis (to identify mutations) at the Gray Laboratory (Partner No. 4).

4. To use the HPV transfected immortalised human keratinocyte cell line to attempt to develop a method for quantifying tumour production in nude mice in relation to cells initially irradiated, initially at risk and at risk at any time post-irradiation.

III. Progress achieved including publications

Referred Publications

1. Harney, J., Mothersill, C., Seymour, C.B. and Murphy, D. p53 Levels in Urothelial Tissues and Cultures from Normal and Bladder Cancer Bearing Patients. Brit. J. Surgery (In press).

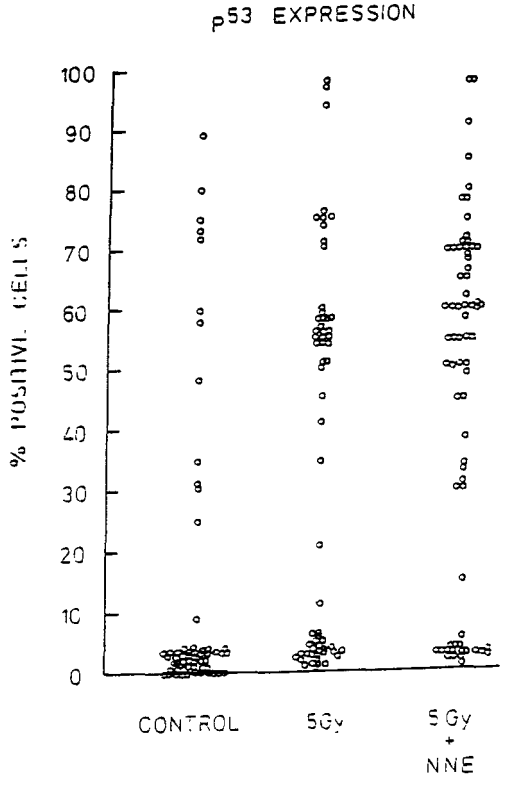
2. Mothersill C., Seymour, C.B., Harney, J. and Hennessy, T.P. Expression of High Levels of p53 and of cmyc in Cultured Human Urothelial Tissue following Carcinogen Challenge using ^{60}Co Irradiation. Radiat. Res. (In press).

3. Mothersill, C., Harney, J. and Seymour, C.B. Induction of Stable p53 Oncoprotein and of cmyc Overexpression in Cultured Normal Human Urothelium by Radiation and N-Nitrosodiethanolamine. Radiat. Res. (Accepted subject to revision).

4. Lyng, F., Mothersill, C. and Cottell, D. Ultrastructural Changes in Human Urothelial Cultures Following Irradiation. Scanning Microscopy Int. (In press).

Achievements

Tissue was cultured from sixty normal donors and 11 cancer-bearing individuals, 22 bladder cancers were cultured as positive controls. Cultures were exposed to gamma or beta radiation in the range 0.1 - 5 Gy (mostly 5 Gy) 24 - 48 hours after explantation.. Two weeks after irradiation, cultures were stained for p53 (P-240 and D07), cmyc (9E10) and EGF_R (2E9). Positive cells were quantified per cell present and per cell initially exposed.



Results for p53 for normal donor cultures are shown on Fig. 1 and they confirm preliminary indications that considerable individual variation exists between donors in susceptibility to induction of stable p53. cmyc induction was very common in all patients. EGF receptor was also up regulated in growing cell populations whether pre- or post-irradiation. Bladder cancer cultures and normal urothelium from cancer-bearing individuals were strongly positive.

A weak positive correlation was found between cell growth (determined by counting $Ki67^+$ cell number) and percentage of stable p53 positive cells in normal controls ($R^2 = 0.76$), but after irradiation, this correlation broke down. This is not surprising in view of the number of conflicting p53 related effects such as cell death, stimulation of proliferation and repair which occur in the cell post-radiation.

High cmyc expression was always strongly associated with cell growth (R^2 = 0.88), but in post-irradiation populations the protein was associated with both growing and apoptotic cells. This supports recent reports in the literature that cmyc determines both proliferation and apoptosis in cycling cells and its ultimate function is dependent on environmental signals and checkpoint genes such as p53, bcl-2 and the cd kinases.

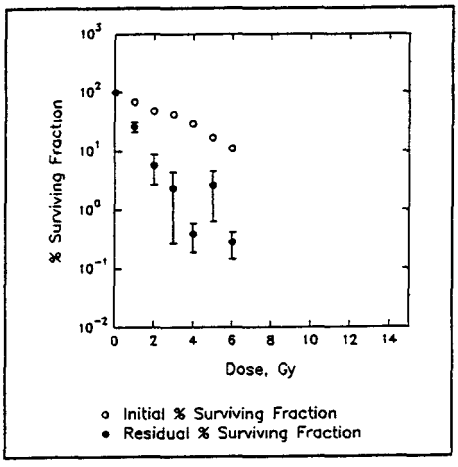
Samples selected from low and high p53 control tissues pre- and post-irradiation have been accumulated for sequence analysis, at the Gray Laboratory, London, of p53 exons 5 - 9 for mutations. It is considered unlikely that this will yield evidence of mutation since the frequency of aberrant p53 protein expression is high. Rather, it is likely that the aberrant protein arises through post-translational conformational change involving perhaps phosphorylation.

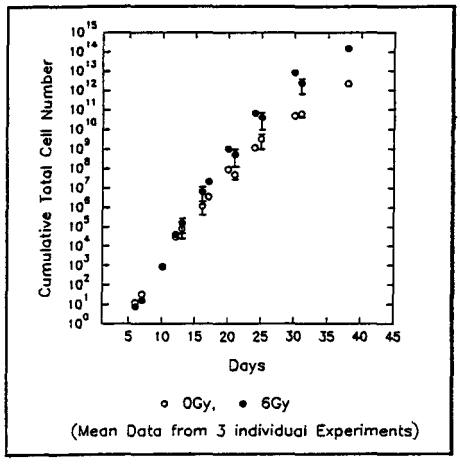
Defective protein in the absence of mutation has been detected in a cancer family and also in a series of Barrett's oesophagus patients. The effect is of particular interest as it is easier to induce with radiation in some patients than in others, and may be indicative of a phenotype which is radiosensitive or which expresses post-translation instability.

Because of the short life-span of human normal epithelial cells in culture and our ignorance of the steps needed to convert radiation initiated cells to transformed cells capable of producing tumours, the Dublin Laboratory, in collaboration with St. Andrew's University, is also looking at HPV transfected human keratinocytes in an attempt to induce end-steps in carcinogenesis by radiation. This line is also being used to quantify the relationship between the number of cells exposed to radiation initially and their clonal progeny existing at the time of injection into nude mice (often up to 3 months and numerous sub-cultures occur between exposure and injection).

The results showed that this cell line is unsuitable for radiation transformation studies. Tumours occurred in unirradiated controls and although these were histologically "less neoplastic", the line is obviously not ideal to use. The clonal expansion data is shown on Fig. 2a and the initial and residual survival of irradiated progeny on Fig. 2b.

Correlation of these data gives a time related factor quantifying cell loss of approx. 15% loss per cell doubling which is constant over the dose range 1 - 6 Gy. The use of this cell loss factor permits the number of clonal progeny to be calculated for the cell line if the time post-irradiation and the doubling time of the cell line is known are known.





Objectives for the Next Reporting Period

1. To focus on the initial response of the irradiated cell - specifically what factors determine whether it repairs faithfully, commits to apoptosis or transforms/mutates. (In collaboration with Inst. Molecular Genetics, Prague).

2. To look at p53 protein expression/induction in normal patients from known radiosensitive groups (e.g. At heterozygotes) or who have other systemic cancer risk factors, e.g. heavy smokers, post-organ transplant or advanced age. This should help to verify the suspected link between individual patient variation in case of p53 induction by radiation and subsequent cancer risk. (In collaboration with Gray Laboratory, London).

3. The methodology used to establish the relationship between cells irradiation and clonal progeny tested for tumourigenicity in nude mice will be applied to HaCaT and HT-ori cells. (In collaboration with C.E.A. France and St. Andrew's University).

PROGRESS REPORT EC CONTRACT NO. F13P-CT93-0067

Head of project 2: Dr. Andrew Riches

II. Objectives for the reporting period.

1. To compare the dose effect relationship for survival and transformation of the human urothelial line SV-HUC-1.

2. To compare the dose effect relationship for survival and transformation of the human thyroid epithelial cell line HT ori-3.

3. To compare the transformation responses of human thyroid epithelial cells exposed to low LET (gamma) and high LET (alpha particle) radiations.

4. To investigate the expression of mutant p53 in irradiated human epithelial cell lines.

III Progress acheived including publications.

Transformation studies using human epithelial cells have been carried out by Dr. Andrew Riches and Dr. Peter Bryant at the School of Biological & Medical Sciences, University of St. Andrews

As carcinomas are the commonest cancers in man, we have focussed on human epithelial cell systems for studies on radiation induced transformation. While human epithelial cells have been transformed by chemicals in vitro. It has proved difficult to develop suitable human epithelial cell systems which can be transformed with radiation. We have used a number of human epithelial cell lines to investigate this problem.

1. Human urothelial cell lines

A number of urothelial cell lines (SV-HUC-1, BC16 and NT11) which had been immortalised following SV40 transfection were kindly provided by Catherine Reznikoff (Department of Human Oncology, Wisconsin, U.S.A.). These cells grow as epithelial sheets in vitro and are reported to be nontumorigenic in athymic nude mice. The lines stained positively for human cytokeratins and for large T antigen and also contained stabilised wild-type p53 antigen. Cells were exposed to gamma irradiation in vitro and passaged following irradiation to maintain the cells in a proliferative state. Following culture for a further 6 to 8

weeks the cells were harvested and transplanted to athymic nude mice. Foci were observed in the nude mice. These cells stained positively with human cytokeratin antibodies but were difficult to rederive as cell lines. Further groups of cultures were irradiated a number of times and following irradiation and passaging were then maintained as a confluent culture. Following periods in excess of 6 to 8 weeks following culture, foci appeared in the culture flasks in the form of papillomas. These have been transplanted to nude mice and have not produced tumours to date. Further groups were treated with methylcholanthrane and exhibited a similar morphological change. The cloning efficiency of cells following irradiation and following chemical carcinogen treatment was increased compared to the untreated cells.

While the urothelial cell lines have shown some promising results it is not yet clear that they will produce a quantitative assay system suitable for studying dose-effect relationships.

2. Human keratinocyte lines.

A human keratinocyte line immortalised by transfection with a human papilloma virus 16 recombinant plasmid construct was used (kindly supplied by Dr.DiPaolo). These cells were irradiated in Dr. Mothersill's laboratory in Dublin and following passaging were transplanted to athymic nude mice at St.Andrews. The cells formed tumours in the mice. However, control unirradiated cells in our hands were also tumorigenic. The growth and morphological characteristics of these tumours derived from irradiated and unirradiated cells are being compared.

While there are some encouraging differences in the tumours derived from irradiated cells and controls, this does not look to be a suitable sytem to exploit for radiation-induced transformation studies.

3. Human thyroid epithelial cells

A human thyroid epithelial cell system has been investigated. The cells stain with antibodies to human cytokeratins. Cells transplanted to athymic nude mice were nontumorigenic at low doses. Cells have been exposed to gamma-radiation before transplantation to nude mice. These mice are currently being screened and 34 tumours have been observed so far. Two of these tumours have been repassaged in vitro to rederive tumour lines. These cells are tumorigenic following transplantation to nude mice. The cells stain positively for human cytokeratins , do not express mutant p53 as measured by

Western blotting and have a human karyotype. In vitro growth rate of these cells is increased compared to the control line. DNA fingerprinting has been used to check that the transformed line was derived from the parent normal thyroid line.

Experiments currently in progress indicate that the human thyroid cells can be transformed following a single exposure to alpha particle irradiation (in collaboration with Dr. D. Goodhead, Harwell).

This epithelial system looks very promising for in vitro/in vivo studies of radiation carcinogenesis. Experiments are in progress to refine the appropriate conditions under which these cells can be transformed and to look at alternative end-points.

Publications.

O'Reilly, S., Riches, A., Bryant, P., Mothersill, C. & Seymour, C.
" Quantification of survival and transformation of progeny of irradiated HPV transfected human keratinocytes. "
International Journal of Radiation Biology (in press)

0'Reilly, S., Mothersill, C., Seymour, C. Riches,A. & Bryant, P.
" Determination of the number of surviving cells available for transformation following irradiation. "
International Journal of Radiation Biology (in press)

Herceg, Z., Bryant, P & Riches, A.
" Radiation-induced transformation of an immortalised human thyroid epithelial cell line. "
International Journal of Radiation Biology (in press)

Riches, A., Bryant, P., Armitage, M., Herceg, Z. & Wang, H.
" Radiation-induced and spontaneous transformation of immortalised human epithelial cell lines. "
International Symposium on Molecular Mechanisms of Radiation and Chemical Carcinogen-Induced Cell Transformation, Michigan, 1993

Head of project 5: Dr Luccioni

II. Objectives for the reporting period

The objective for this period was to begin to accumulate biological material in order to compare cells at different subculture times after chemical treatment or radiation exposure.

This work consisted in:

-treating the animals;

-initiating and maintaining cells in culture;

-beginning cell characterization.

Since protocols had already been described, chemical transformation was performed first.

III. Objectives for the next reporting period

-Pursue cell culture of gamma irradiated and control cells until they reach passages where changes occured in chemically treated cells.

-Characterize the cells at different intermediary passages to study the changes occuring and their chronology using endpoints defined in the contract program.

brains from fetus or newborn rats were isolated and cell cultures initiated as previously described. In parallel, brains were isolated from 2 pregnant rats at day 21 of gestation and day 5 after birth and cell cultures initiated, to serve as control for the 2 models of glial cells transformation. For all these samples, cell culture techniques and treatment (TPA and acetone) are as previously described. Cells are still at early passages with apparent characteristics similar to chemically treated cells at the same stage. No stable chromosomal aberration is yet observed.
In parallel, western blot techniques to study GFAP, vimentine and protein S-100 expression have been set up.

Study of *in vitro* transformation is a long term project, representing a lot of cell culture time. This period corresponds to an accumulation of biological material for further studies.

IV. Progress achieved including publications

The great latency in carcinogenesis studies does not allow to report a lot of results but the first data collected are exposed.

This work started about 10 months ago. Pregnant rats at day 18 of gestation time were injected intraperitoneally with ethylnitrosourea (ENU), 50 mg/kg. Three days later, brain from fetuses were isolated, mechanically dissociated and cell cultures were initiated. This step was performed under Dr H. Coffigny's supervision. Cells are continuously treated either with phorbol myristate acetate (TPA, 1 ng/ml) or with the solvant acetone (0.01% final) as control. Cells are trypsinated and subcultured when they reach confluency. Medium is changed twice a week.
At the beginning, cell population was heterogeneous, but with successive passages, an homogeneous cell type was selected. Pictures are regularly taken to evidence the changes in morphology.

The first passages took a very long time, but progressively the time interval between passages decreased. Now the cells exhibit characteristics of an established cell line.

Caryotypes are performed with the R-banding technique, routinely used in our laboratory. At least, 10 metaphases were scored and analysis is performed under Dr B. Dutrillaux's supervision. Caryotypes, at early passages were normal. Caryotypes from normal rat skin fibroblasts were used as control. A caryotype at passage 20 for cells treated with TPA showed 2 populations, one being near tetraploïd. At passage 40, the caryotype is abnormal. The cells are hypotetraploïd with about 60 chromosomes. The chromosomes 1, 3 and 11 are rearranged and there are few markers. These modifications can indicate that a transformation process has occured.

During the subculture time, at different passages, cells were regularly frozen with glycerol and stored in liquid nitrogen for further studies.
Tumorigenicity was tested on cells treated by TPA or acetone at passages 25 and 30, by injecting them in the temporal brain region of 3 or 4 rats being 4 weeks-old. So far, 2 rats died, authopsy showed absence of brain tumor. The tests on cells at later passages are under way. Clonogenicity was also tested by culture of cells treated by TPA or acetone on agar, first at passage 35, then at passage 50. Colonies were observed at passage 35 only for cells treated with TPA, and at passage 50 for the 2 cell lines.

Few months later, pregnant rats were irradiated at day 14 and day 20 of gestation time, with cobalt 60. The dose was 0.5 Gy per day for 7 days. 3 days later,

Head of project: Dr. M. Martin

II. Objectives for the reporting period

The objective of the first period of the contract was to define culture models that might be relevant for cell transformation studies of the skin. As there is at this date no available model to study the whole process of cell transformation in human cells, we performed experiments on two cellular models. The first model is normal keratinocytes, which allow to study the early steps of cell transformation in cells expressing a normal p*53* gene. The second model is immortalized, non tumorigenic keratinocyte cell lines, which have mutated p53 gene and may allow to study the last steps of cell transformation after γ irradiation. In all of these experiments, we wanted to focus our attention on the p*53* gene at the level of transcription (p*53* mRNA) and translation (p53 protein), because a key role is currently proposed for this protein in controlling the G1 growth arrest after exposure of the cell to radiation, and consequently the accumulation of genetic lesions leading to cell transformation.

III. Progress achieved including publications

1/ Normal human keratinocytes

Normal keratinocytes allow to study the early steps of cell transformation in cells expressing a normal p*53* gene. Thus, normal human epidermal keratinocytes were isolated from human mammary skin (mammoplasty procedure) or from human abdominal skin obtained after surgical excisions.

Normal keratinocytes in the reconstituted skin model: The reconstituted skin is composed of an epidermal sheet (human keratinocytes) which stretches on a dermal matrix (human fibroblasts). This model accounts for the epidermal maturation (proliferation and differentiation) program of human keratinocytes, which is regulated by stromal cells.

We have carried out irradiation of this reconstituted skin in order to evaluate radiation-induced alterations of the p53 gene expression. The irradiation was carried out using a ^{60}Co source at a dose-rate of 0.2 Gy / min. At different times post-irradiation (3 h, 1 day, 1 week), samples were snap-frozen in liquid-nitrogen and vertical sections were made; RNAs were also recovered from the dermis or epidermis compartment. As evidenced by immunofluorescence stainings, we

observed an early (3 h post-irradiation) and transient induction of p53 expression, likely due to an enhanced stabilization of the protein. Studies at the mRNA level were performed and they showed little variations of the *p53* mRNA level in keratinocytes and no modification in the fibroblasts.

Normal keratinocytes in monolayer: In monolayer, keratinocyte culture was easily achievable. However, these primary cells could not be maintained after 4 passages in culture. We are currently comparing the induction of the p*53* gene obtained in poor differentiation culture conditions (monolayer) with high differentiation culture conditions (skin equivalent).

2/ Human immortalized keratinocyte cell lines

Immortalized, non tumorigenic keratinocyte cell lines may allow to study the last steps of cell transformation after γ irradiation. Thus, Rhim (1990) succeeded in transforming the RHEK-1 cell line with gamma rays. We wanted to define the morphology, proliferation, genetic stability and gene expression for oncogene markers of HaCaT cell line.

HaCaT in the reconstituted skin model: These human cells have been isolated from the normal skin of a cancer patient. They became "spontaneously" immortalized but remained non tumorigenic. (Boukamp et al 1988). The status of the p53 gene has recently described as abnormal, as HaCaT cells have mutations for both p53 alleles (Lehman, 1993).

At the LRA, this cell line was cytogenetically characterized in collaboration with C. Luccioni. These cells were near tetraploid, exhibited numerous chromosome anomalies and were genetically unstable in culture. However, in the reconstituted skin, the HaCaT were still able to develop an epithelium.
HaCaT cells exhibited an abnormal p*53* gene expression. A high expression was demonstrated both at the transcriptional level with cDNA probes for *p53* and at the protein level using immunoprecipitation and immunofluorescence on histologic slides in the skin model. A cytoplasmic localization of the p53 in HaCaT cells could be ascribed to the mutational pattern of the p53 gene, which was also revealed by its electrophoretic pattern after immunoprecipitation (appearance of Hsp70 - p53 complexes).

Following our strategy, we have irradiated the skin equivalent model (HaCaT cells and normal human fibroblasts) 3 weeks after gelling, when epithelium was stratified. We observed a high induction of p53 protein at doses as low as 0.5 Gy. This induction remains stable for 7 days following irradiation. We found no increase in p*53* mRNA level up to 2 Gy, but a strong induction of p*53* mRNA level with higher doses (8 Gy).

HaCaT in monolayer: In monolayer, HaCaT cells retained an epithelial morphology. Surprisingly, irradiation of HaCaT in monolayer leads to a dose-dependent increase in the p*53* mRNA level 3 h post-irradiation, with graded doses of γ–rays up to 8 Gy. We have also performed immunoprecipitation procedure against p53 protein (with the pAb122 hybridome supernatant). It appeared a sustained stabilization of the protein 3 h post-irradiation at 2 Gy.

3) Conclusion

Most experiments published on p*53* and the cellular response to gamma radiations concerned immortalized cells belonging to the hematopoietic lineage. We have developed two cellular models which allow to study the radiation response of the skin.

Normal keratinocytes and fibroblasts allow to study the early steps of cell transformation in cells expressing a normal p*53* gene. The radiation response can be studied in poor differentiation (monolayer) and high differentiation (reconstituted skin) culture conditions. In these cells, gamma rays induce the p53 protein with a post-transcriptional regulation.

In HaCaT cells, which have p53 mutations, the endogeneous p53 expression was high and abnormal. Furthemore, the induction by the gamma rays exhibited uncommon regulations, particularly an induction at the transcriptional level, which has never been described for cells belonging to the hematopoietic lineage. As certain p53 mutants loose their antiproliferative activities and acquire oncogenic properties, then this sustained induction of the p53 protein might be one of the key requirements for transition towards neoplasia. In conclusion, HaCaT cells might be a good model to study the last steps of transformation in cells with an abnormal p*53* gene.

In conclusion, although non tumorigenic, the HaCaT cells have probably passed through several genetic events necessary prior to cell transformation, and may give rise to complete neoplastic transformation after gamma irradiation.

4) Publications

- **Martin M., Lefaix J-L, Crechet F., Pinton P., and Daburon F.** Temporal modulation of TGF-ß1 gene expression in pig skin and muscular fibrosis after ionizing radiation. Radiation Research, 1993, 134, 63-70.

- **Lefaix J-L, Martin M, Tricaud Y and Daburon F.** Muscular fibrosis induced after pig skin irradiation with single doses of 192 Ir gamma rays. The British Journal of Radiology, 1993, 66, 537-544.

- **Martin M., Pinton P., Crechet F., Lefaix J-L, and Daburon F. Preferential induction of c-fos** proto-oncogene during the immediate early response of pig skin to gamma rays. Cancer Research, 1993, 53, 1-4.

- **Martin M.** Early induction of transcription factors by ionizing radiation: example of the skin radiation response. In: Molecular mechanisms in radiation mutagenesis and carcinogenesis, K. H. Chadwick (ed), in press.

Communications.

- (O) **Martin M., Crechet F., Pinton P., Lefaix J-L.**, 1992. Preferential activation of the c-fos protooncogene during skin radiation response.24th Annual Meeting of the European Society for Radiation Biology, Erfurt (D) 10-1992.
- (P) **Martin M, Lefaix J-L., Pinton P., Crechet F. Daburon F.** Expression du facteur de croissance TGFb dans la fibrose musculaire. 5ème Colloque National sue les Maladies Neuromusculaires, Strasbourg (F), 06 1993.
- (P) **M. Martin and D. Biard**. Early upregulation of the *p53* tumor suppressor gene in human epithelial cells irradiated in the skin equivalent model, 1993. Mackinac Island International Symposium, USA, 09 1993.
- (P) **Biard D. and Martin M.** Induction of the p53 gene in normal humam keratinocytes irradiated in the skin equivalent model. Annual meeting of the ECTS, Rennes (F), 07 1993.

Head of project 7: Dr. Arrand

II. Objectives for the reporting period

1. To optimise the conditions for the determination of the status of the p53 gene in the untransformed cell lines being used by each of the participating groups to study radiation-induced transformation.

2. To set up a low dose rate radiation facility for use by all groups.

3. To characterise the radiation response of our untransformed human lung epithelial cell line (L132) at the cell survival level using computerised microscopy (DMIPS).

4. To similarly characterise the untransformed cell lines from the other groups.

5. To analyse expression of p53 and other potential transformation marker proteins in L132 cells.

6. To search for new potential transformation markers.

7. To set up and make available both high and low LET radiation facilities for all participating groups.

III. Progress achieved including publications

1. A p53 cDNA clone and PCR primers for exons of the p53 gene have been obtained from the Human Genome Mapping Project. Conditions have been optimised for their use in Southern/ northern blots and for amplification/ mutation detection respectively.

2. We have established a low dose rate ^{60}Co γ-ray source at 37° delivering a minimum dose rate of 0.001 Gy/ h. We are currently using this facility to irradiate L132 cells and will shortly be receiving cells from the Dublin group for low dose rate exposure.

3. We have extensively characterised the L132 cell line at the cell survival level using our computerised microscope (DMIPS) and have found a 0.5 Gy inducible response (Singh et al., 1993, Int. J. Radiat. Biol., *In press*)

4. This analysis is being extended to cell lines from the other participating groups with a study of the HuCAT cell line from St. Andrew's already completed.

5. We are analysing expression of p32 and p34cdc2 kinase proteins in L132 cells.

6. We are looking for novel transformation markers at both the protein and mRNA levels.

7. We have made our high- and low-LET sources available to all participating groups.

Progress on Contract F13P-CT930067; project 7

The major progress made over the past few months has been in thoroughly characterising the normal human cell line which we propose to use for our radiation transformation experiments. This cell line (L132) is a near diploid bronchial epithelial line with a limited life span. It is not virally immortalised and is contact inhibited. Preliminary experiments have shown that it metabolises, and can be transformed by, benzo(a)pyrene and that following exposure to ionising radiation, its life span is increased and it forms non-contact inhibited colonies.

Our first objective was to establish the response of this cell line to low dose X-irradiation. Cell survival measurements were made using a Dynamic Microscope Imaging Processing Scanner (DMIPS) which allows single cells to be located accurately, their positions recorded and then these positions revisited after an appropriate incubation period at 37°. Surviving cells were identified by their ability to produce a colony of more than 50 cells. For irradition, cells were exposed as monolayers to X-rays (0.05 - 4 Gy) generated from a Pantak unit operating at 240 kVp. A dose rate of 0.49 Gy min^{-1} was used for these irradiations. Control samples were sham irradiated under similar conditions.

Figure 1 shows the survival data for these cells generated in 10 independent experiments plotted as the mean survival at each dose. The lines represent the fits to the complete data set of an "inducible repair" model (solid line) and to the data for doses ≥ 2 Gy of the linear quadratic model (dotted line). The dashed line shows a linear quadratic curve based on the value of α_r obtained from the fit of the inducible repair model (for mathematical defination of the models, see Singh et al., 1993, Int. J. Radiat. Biol., *in press*).

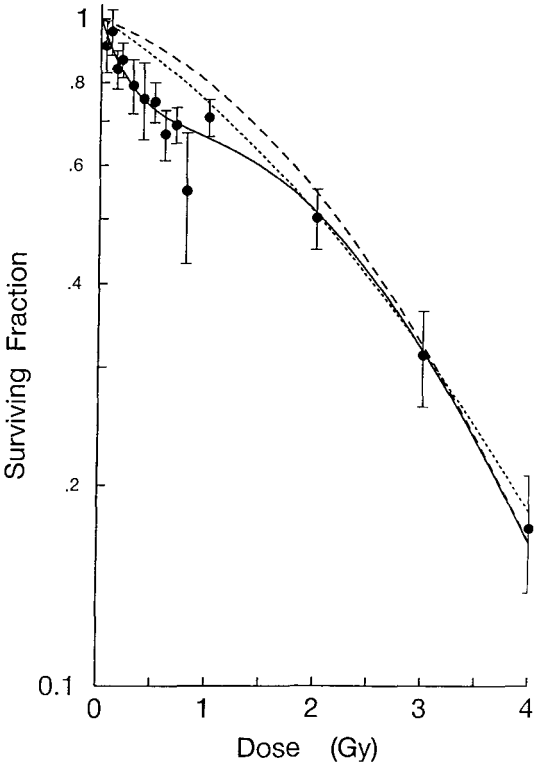


Figure 1. Survival of L132 cells as measured on the DMIPS

The very accurate data generated at low doses using the DMIPS shows that at doses greater than 2 Gy, the L132 cells show a classical survival curve that fits the linear quadratic model. However, at lower doses it is clear that there is a deviation from this fit. At doses of less than 0.2 Gy, there is a region of hypersensitivity to X-rays. At doses between 0.4 - 0.6 Gy there is a transition region where cells become more radioresistant and finally, above 1 Gy, the survival curve returns to the linear quadratic expectation. The biphasic nature of the curve is suggestive of the presence of an induced radioprotection mechanism which is triggered by a dose of approximately 0.5 Gy.

We have recently extended this study to include the SV-HUC-PC cells from the St. Andrew's group. The data is shown in Figure 2 and a very pronounced inducible radioprotection is seen, again at doses of around 0.5 Gy. We have further observed that cells from some radiosensitive tumours and SV40-immortalised fibroblasts from ataxia telangiectasia group D lack this inducible response. We therefore propose that, in cells possessing the inducible response, a low dose exposure protects against the killing, mutational and tranforming effects of subsequent higher dose exposures and that molecular analysis of this effect might provide us with new molecular markers for radiation-induced transformation of human cells.

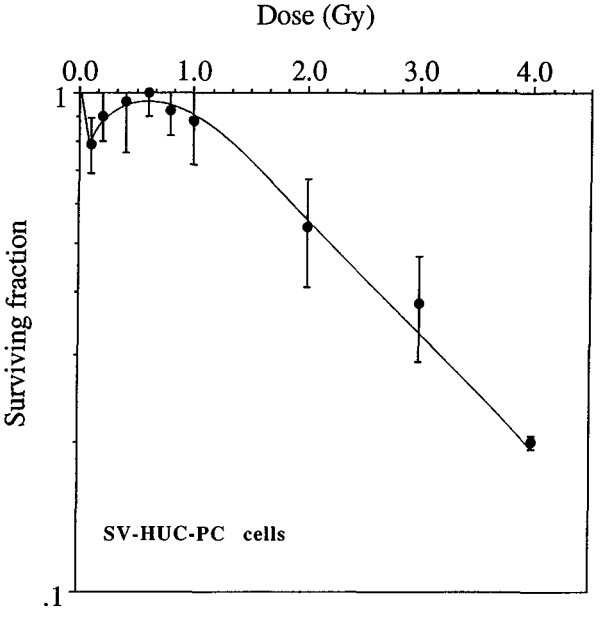


Figure 2. Survival of SV-HUC-PC cells as measured on the DMIPS

The involvement of genes, known to be induced at high doses of radiation, in this low dose inducible effect have been analysed at both the protein and mRNA levels. p53 protein levels are not increased in the L132 cell line following doses of 0.2, 0.5 or 1 Gy. 2-D gel analysis of proteins from 0.5 Gy irradiated L132 cells suggests that there are many upregulated and downregulated proteins but that at least 2 examples of proteins post-translationally modified following low dose exposure exist. The molecular weights of these 2 proteins are 35 kda and 65 kda. These are being further characterised by microprotein sequencing. We have shown that the X-ray inducible response in L132 cells does not require *de novo* protein synthesis. It is therefore possible that the response is initiated by the modification of existing proteins which in turn initiate a cascade of reactions leading to the observed effects.

Northern blot analysis of mRNAs from 0.5 Gy irradiated L132 cells show that cdc2, β-PKC, c-myc, c-fos, TK, AP-endonuclease, metallothionein and alkyltransferase, which have been shown to be induced at high doses, are not induced at 0.5 Gy thus ruling out their involvement in our observed low dose effect. The induced radioprotection may therefore be initiated by a novel signalling cascade. We are investigating this further by isolating cDNAs by differential screening of a library from 0.5 Gy induced L132 cells with irradiated and non-irradiated cell mRNAs and have, thus far, isolated one down-regulated gene. This, and others identified in our differential screens will be assessed as potential ionising radiation transformation markers in L132 cells and the cell lines being used by the other participating groups in this programme. Expression of these genes will assessed in unirradiated cells and to those exposed to low doses or at low dose rate in our ^{60}Co facility at various times during potential oncogenic transformation and will be added to the data which will be accumulated on the status and expression of the p53 gene in cells exposed to these treatments.

Progress Report

Contract: FI3P-CT920005 **Sector:** B14

Title: Radiation-induced genetic effects in mammals and the estimation of genetic risks in man: a concerted approach using theoretical, epidemiological, cytogenetic, biochemical and molecular methods.

1)	Sankaranarayanan	Univ. Leiden
2)	Tease	MRC
3)	Jacquet	CEN/SCK Mol
4)	Streffer	Univ. Essen

I. Summary of Project Global Objectives and Achievements

Analysis of data on human congenital abnormalities (CAs) and of evolutionary models on the maintenance of quantitative traits in populations has been completed. A first inquiry comparing the extent to which naturally-occurring genetic diseases can serve as a baseline for genetic risk estimation for ionizing radiation and chemical mutagens has also been completed. Some progress has been achieved in the development of models for radiation risk estimation for congenital abnormalities in man. Molecular genetic studies of isolated cleft lip ± cleft palate in humans suggest that the gene is not linked to TGF-alpha marker on chromosome 2 in contrast to the findings reported by Ardinger et al (Am. J. Hum. Genet. 45, 348-359, 1988) (K. Sankaranarayanan).

The suitability of a newly-developed protocol for *in situ* hybridization of mouse epididymal spermatozoa was assessed in a pilot study on X-ray-induced sex-chromosomal aneuploidy in male germ cells. In epididymal spermatozoa collected 30 days post-irradiation (2 Gy) there are indications for an increase in the incidence aneuploid spermatozoa suggesting that the technique can be used in radiation studies (C. Tease)

Progress has been made in improving the techniques for *in vitro* culturing of guinea pig oocytes for radiation cytogenetic studies. The initial results of X-irradiation (2 Gy) of immature oocytes (mice irradiated within the first two days after birth and oocytes sampled one year later) show that translocations and other chromosomal aberrations can be induced in this germ cell stage most relevant for genetic risk assessment (P. Jacquet).

The effects of low dose-rate gamma irradiation of female mice (0.18 mGy/min; total doses of 0.7 and 1.4 Gy) on fetal lethality (FL) and on the incidence of malformations (M) in living fetuses were studied. The fetuses analysed were from conceptions which occurred 5 to 9 (FL) or 5-7 weeks (M) post-irradiation. At the dose of 1.4 Gy (i) "total litter loss" increased from about 40% in the controls to 70-80% in weeks 6 and 7 and to 100% in weeks 8 and 9 and (ii) in weeks 5-7, malformed fetuses showed a significant increase from about 1.4% in controls to 17% (C. Streffer).

Head of project 1: Dr. Sankaranarayanan

II. Objectives for the reporting period

1. Analysis of our current knowledge on human common congenital abnormalities and development of suitable models for radiation genetic risk estimation for this class of diseases;
2. Initiation of molecular studies on the genetic basis of isolated cleft lip ± cleft palate in humans and
3. A comparison of the extent to which naturally-occurring Mendelian diseases can serve as a frame of reference for genetic risk estimation for radiation and chemical mutagens.

Objectives for the next reporting period

1. Completion of the molecular studies on the genetic basis of cleft lip ± cleft palate in humans;
2. Application of the mathematical models developed for assessing the effects of an increase in mutation rate on the prevalence of congenital abnormalities in man;
3. Continuation of analysis of the molecular nature and mechanisms of origin of mutations involved in human Mendelian diseases (further to Sankaranarayanan, Mut. Res 258, 3-122, 1991) and
4. Initiation of analysis of our current knowledge on adult-onset multifactorial conditions to provide a basis for the development of suitable models for radiation genetic risk estimation.

III. Progress achieved including publications

Congenital abnormalities: theoretical studies (Item II-1).

An in-depth analysis of data on the epidemiological aspects of congenital abnormalities (CAs) in man, of the multifactorial threshold model (MTM) and other models which have been proposed to explain their transmission characteristics and recurrence risks in families and of evolutionary models on the mechanisms of maintenance of quantitative traits in the population has been completed. Most of the data on CAs used for this purpose are those collected in Hungary and British Columbia, Canada.

CAs which affect an estimated 6% of all livebirths are aetiologically heterogeneous. The majority do not follow Mendelian transmission patterns, but do "run" in families. the MTM is an extension of genetic principles developed for quantitative traits to "all-or-none" traits. In its simplest formulation, it assumes the existence in the population of an underlying Normally (Gaussian) distributed "liability" (which is due to numerous genetic and environmental factors acting additively and of a "threshold" beyond which the individual is affected. For most CAs, the nature of these factors remains

unknown. Other models assume fewer causal factors although, again, these remain to be identified.

The question of how considerable amount of heritable variation for most quantitative/polygenic traits has come to exist is a long-standing one in evolutionary population genetics. Models postulating that its existence is consistent with a balance between recurrent mutation and stabilizing selection or suggesting possible operation of other mechanisms have been published in the literature.

In the absence of knowledge on mechanisms responsible for the stable prevalences of CAs in the population (but which is required to predict the consequences of an increase in mutation rate on their prevalences), it is necessary to (a) adapt and use concepts derived from quantitative and evolutionary population genetics and (b) to examine how sensitive the predictions of these new models are to the assumptions used, and how consistent they are with biological realities.

The MTM as originally conceived and applied to CAs in man did not incorporate mutation and selection, the primary mechanisms which are believed to be responsible for the maintenance of heritable variation for quantitative characters. In the modified MTM which has been developed in collaboration with Dr. Tusnady (Budapest, Hungary), Dr. Yasuda (Chiba, Japan) and Dr. Chakraborty (Houston, USA), gene frequencies per se do not enter the picture; instead, it considers a starting liability distribution and estimates values of mutation and selection consistent with prevalences in population and in first degree relatives of specific CAs on the basis of empiricl data. Using these as input values, it inquires into the consequences of doubling of mutation rate. Our first results suggest a permanent doubling of the mutation rate will eventually lead to a doubling of the frequencies of CAs, but the approach to equilibrium is very slow; the expected increases in the first few generations are very small.

Molecular studies on the genetic basis of isolated cleft lip ± cleft palate (CL ± CP)(Item 2, (II)). The rationale for using the above condition for our work comes from the studies of Ardinger et al (Am. J. Hum. Genet 45, 348-353, 1989) who reported an association between transforming growth factor alpha (TGF-alpha) on the short arm of chromosome 2 (p13) and CL ± CP. This study was population-based. The authors speculated that either the TGF-alpha gene or sequences close to it may contribute to the development of CL ± CP in a proportion of the cases. In more recent studies with 12 multiplex families, Hecht et al (Am. J. Hum. Genet 49, 682-686, 1991) could not obtain evidence for tight linkage of the putative CL ± CP locus to TGF-alpha but indicated that this condition may be a heterogeneous group of disorders and that additional studies are warranted in other multiplex families.

In our work, multigenerational families with more than one affected case (usually between 2 and 5) were collected through collaboration with the Hungarian Congenital Malformation Registry Centre. All cases were medically confirmed and the status of the CL (uni- or bilateral) and the involvement of the palate were registered. Blood samples were obtained from members of 7

affected families (61 individuals). DNA extracted following standard procedures were used in molecular studies using conventional polymorphic (RFLP) markers. The restriction enzymes used included RsaI, TaqI and BamHI. These studies have so far not provided any significant evidence for linkage such as that found by Ardinger et al mentioned above.

The extent to which naturally-occurring Mendelian diseases can serve as a frame of reference for radiation risk estimation for radiation and chemicals: a comparative study (Item 3 (II)). This inquiry which took into account molecular nature of spontaneous mutations underlying human Mendelian diseases, the nature of radiation and chemically-induced mutations in experimental systems and the mechanisms involved permits the following two tentative conclusions: (i) naturally-occurring Mendelian diseases do not constitute an entirely adequate frame of reference for risk estimation and current radiation risk estimates for Mendelian diseases are likely to be conservative, but provide an adequate margin of safety for radiological protection and (ii) chemical mutagenesis is adduct-driven with its own specificities; when these chemical specificities are considered in conjunction with those of mutational sites in most disease-related genes, it would seem that such mutations may be induced with a probability higher than that in the case of ionizing radiation. Further, one can argue that there must be some overlap in specificities between spontaneous and chemically-induced mutations, but its extent is at present unknown.

Publications (including papers in press)

Sankaranarayanan, K (1992) Ionizing radiation, genetic risks and radiation protection. In Proc. Int. Conf. Radiation Effects and Protection, Mito, Japan, pp 65-70, Japan Atomic Energy Research Institute, Tokyo, Japan.

Sankaranarayanan, K (1992) Estimates of genetic risks of exposure to ionizing radiation and their use in radiation protection. J. Radiol. Prot 12, 129-136.

Sankaranarayanan, K (1993) Ionizing radiation, genetic risk estimation and molecular biology: impact and inferences. Trends in Genet 9, 79-84.

Sankaranarayanan, K (1993) Estimation of genetic risks of exposure to chemical mutagens: relevance of data on spontaneous mutations and of experience with ionizing radiation. Mutation Res., in press.

Sankaranarayanan, K., N. Yasuda, R. Chakraborty, G. Tusnady, A. Czeizel (1993). Ionizing radiation and genetic risks. V. Multifactorial diseases: a review of epidemiological and genetic aspects of congenital abnormalities and of models of maintenance of quantitative traits in populations. Mutation Res., in press.

Karcagi, V., L. Timar, K. Sankaranarayanan, E. Bakker (1993). Linkage studies on Hungarian families with non-syndromic cleft lip and palate. Abstract, Proc. III Biennial Mammalian Developmental Genetics Workshop (8-12 Sept 1993), Bar Harbor, Maine (in press).

Head of project 2: Dr. Tease

II. Objectives for the reporting period

1. To determine a suitable protocol for efficient *in situ* hybridization of chromosome-specific probes to mouse epididymal spermatozoa.
2. To perform a pilot experiment using X-irradiated spermatozoa.

III. Progress achieved including publications

Although the *in situ* hybridization protocol currently in use in this laboratory works well with mouse spermatocytes and testicular spermatozoa, it was found that epididymal spermatozoa gave a low proportion of cells with a detectable hybridization signal. Other investigators have suggested the use of trypsin and/ or dithiothreitol (DTT) as pre-treatments to decondense sperm chromatin to some degree and thus make it more accessible to DNA probes (e.g. Joseph et al (1984) Human Genetics 66:234). Comparison of various treatment regimes showed that a 3 minute pre-treatment with a solution of 0.1% trypsin/ 50mM DTT gave high hybridization efficiency with minimal disruption to sperm head structure. This proved to be the case for repeat sequence probes for the X (probe 70-38X) and Y (probe 353Y) chromosomes.

Results of the pilot X-ray experiment

Young C3H/HeH x 101H male mice were given 2 Gy of acute X-rays; epididymal spermatozoa were collected 6 or 30 days after irradiation. Germ cells in the former sampling interval would have been mature spermatozoa at the time of exposure, while at the latter interval would have been primary spermatocytes. Age-matched, unirradiated males served as controls. Detection of X or Y chromosomes was carried out using the appropriate biotinylated probe and use of the standard avidin-FITC and biotinylated anti-avidin procedure. Systematic scoring of spermatozoa was carried out by traversing the slide using the 100x objective and including all cells that fell within a designated area of the field of view.

The results of the experiment are given in Table 1. In the control, unirradiated males the proportions of cells with either an X or a Y chromosome did not deviate significantly from the expected 50%. At the 6 day interval, both irradiated males had an excess of cells with a single X signal; however, even when the data from both males were combined the excess was not significant ($\chi^2_1 = 1.69$, $P > 0.05$). The results with the Y probe were in agreement with this conclusion; one male had a slight deficiency and the other a slight excess of cells with a single Y signal.

At the 30 day interval, the irradiated male had a marked deficit of cells with either a single X or Y signal. Interestingly, the proportions of cells scored as containing 2 signals was elevated for both probes. The apparently larger increase for the X probe may be an artefact as this probe gave a discrete signal within the cells which made identification of the presence of 2 signals relatively straightforward. In contrast, the Y probe gave a more

diffuse signal which made such judgement more difficult; only those cells in which 2 signals were unambiguously present were included in this category, the ambiguous cells being assigned to the single signal group.

Table 1 The proportions of spermatozoa with an X or Y chromosome from unirradiated mice and from males given 2 Gy of X-rays either 6 or 30 days before sampling.

Treatment group	Proportion of cells (%)			Total cells
	X	XX	-	
control	49.84	0.13	50.03	1583
2Gy/6 day	51.83	0.06	48.11	1588
	51.55	0.06	48.39	1610
control	49.78	0	50.22	1595
2Gy/30 day	47.43	1.29	51.29	1632
	Y	YY	-	
control	50.74	0	49.26	1624
2Gy/6 day	49.08	0	50.92	1628
	50.90	0	49.10	1605
control	49.51	0	50.49	1626
2Gy/ 30 day	48.52	0.46	50.78	1723

The apparent lack of any effect of X-irradiation in the 6 day sample is as expected: the cells would have been mature spermatozoa at the time of exposure and so no alteration in the proportions of cells with one or other sex chromosome would be anticipated. Clearly, this constraint did not apply to the 30 day interval when the germ cells would be primary spermatocytes and have 2 meiotic divisions to undergo. In this instance there was scope for segregation errors, nondisjunction or chromosome loss, to occur. The data tentatively point to an increased incidence of cells without an X or Y chromosome suggesting that the treatment may have increased the rate of chromosome loss. More clear, however, was the elevation of the rate of cells with more than 1 signal. Although it is possible that these cells resulted from nondisjunction of the X and Y chromosomes to produce disomic spermatozoa, a more likely explanation is that the cells were diploid for it was noted that the majority of cells with 2 signals were markedly larger than their near neighbours.

The pilot experiment has confirmed the potential of the protocol to work efficiently when using cells from irradiated animals. More interestingly, it has indicated that it has the potential to detect anomalous sex chromosome segregation following irradiation of

primary spermatocytes.

IV. Objectives for the next reporting period.

1. To carry out simultaneous hybridization of the X and Y chromosome probes thus enabling all cells to be assigned to a particular category.
2. To investigate further the possible induction of diploidy by irradiation through use of a repeat sequence probe for chromosome 8.
3. To carry out a larger scale X-ray experiment to determine whether the preliminary results of the pilot work can be confirmed.

Head of project 3: Dr. Jacquet

II. Objectives for the reporting period

The extension of radiation studies to oocyte systems which are more comparable to the human one than the mouse oocyte, both in terms of sensitivity to killing and nuclear morphology is strongly indicated. Investigations performed in our laboratory showed that the guinea-pig oocytes represents such a system. The work planned for this year consists in two parts: 1) improvement of the techniques (developed earlier in our laboratory) to obtain and culture guinea-pig oocytes *in vitro* and to make cytogenetic preparations; 2) investigating the cytogenetic radiosensitivity of the large resting oocyte by irradiating newborn animals; our priliminary results showed that chromosomal aberrations can indeed be induced in them, in contrast to the situation known for mouse oocytes.

III. Progress achieved including publications

As mentioned above, many efforts were devoted to the improvement of the various aspects of the techniques of *in vitro* culture of guinea-pig oocytes. Although the first results obtained two years ago were encouraging,they suffered important variability. One of the problems which we encountered was the regular presence of a high proportion of second meiotic divisions (up to 100%) among oocytes which had been cultured even for relatively short periods. Thus, extensive investigations were performed last year to test the influence of various parameters on the oocyte maturation. These included :

- the use of various culture media, supplemented or not with EDTA and/or colchicin. In the mouse, EDTA has been shown to exert a stimulatory effect on the cleavage of cultured one cell embryos, which normally should undergo the so-called "two-cell-block". It was thought that the presence of this compound in our culture media could eventually also exert a stimulatory effect on the first meiotic division;
- the culture of the oocytes during progressively shorter times, i.e. 7, 6, 5.5, 5, 4.5, 4, 3.5, 3, 1.5, 1 hours.
- the fixation of the oocytes without preliminary *in vitro* culture
- the use of various media (Yanagimachi, J. Reprod. Fert. 38, 485-488, 1974; Yamada et al., Radiat. Res., 92, 359-369, 1982), with or without Ca^{2+} and Mg^{2+}, and at different temperatures and pH, for collecting oocytes from the ovaries as well as for removing

follicular cells and the zona pellucida before the fixation of the oocytes.
From these studies, it clearly appeared that, under some experimental conditions, the guinea-pig oocyte can resume almost instantaneously the meiotic maturation to the metaphase of the second division, a process which normally requires at least 15 hours in cultured oocytes from all mammals studied so far.
Stringent conditions were defined, which allowed obtention of 100 percent MI after culture of the guinea-pig oocytes during 4 hours. These conditions have now been standardized.

Another factor which retained our attention was the determination of the period of the oestrous cycle (17 days in the guinea-pig) during which the highest number of meiotically competent oocytes could be obtained, since great differences in this respect were also evident. Ovaries at days 8-10 of the oestrous cycle showed the best results.

Finally, many efforts were still achieved in order to control the quality of fixation and spreading of the oocyte chromosomes. In the guinea-pig, this is a particularly difficult step, since the enzymatic treatments to remove the remaining follicular cells and the thick zona pellucida can have a disastrous effect on the quality of the preparations. In addition, the guinea-pig oocyte contains a great amount of yolk globules which can strongly affect the quality of the preparations.

The improvement of these various aspects of the *in vitro* culture of guinea-pig oocytes necessitated prolonged studies until this year. The results obtained during the last months were quite satisfactory, in that the proportion of cultured oocytes which could be analyzed cytogenetically reached sometimes as much as 50% or more.

The cytogenetic investigations which are now in progress concern mainly the radiosensitivity of the immature resting oocyte. For this purpose, female guinea-pigs were mated last year and, during the two first days following delivery (duration of pregnancy: ± 68 days), female progeny (1.5-2 per pregnant female) were irradiated with 1 or 2 Gy of X-rays on the ovaries. Their oocytes are being examined for the presence of translocations and/or other chromosome aberrations, one year after irradiation.
The results will complete preliminary data obtained two years ago with the dose of 1 Gy. Although the number of analyzed oocytes is still low, the results obtained for the dose of 2 Gy already confirm that translocations can be induced in this most important stage of oogenesis.

Publications

P. Jacquet, J. Vankerkom and M. Lambiet-Collier, The female guinea-pig, a useful model for the genetic hazard of radiation in man; preliminary results on the germ cell radiosensitivity in fetal, neonatal and adult animals, submitted for publication.

IV. Objectives for the next reporting period

For the next reporting period, we intend to continue investigations on the radiosensitivity of another stage of oogenesis, i.e. the mature, preovulatory oocyte. First studies on this subject were performed at the beginning of last year, but they were temporarily stopped, due to the difficulties encountered in obtaining good cytogenetic preparations of MI oocytes. For these studies, a dose of 4 Gy had been used and revealed highly detrimental for the oocytes. Doses of 1 and 2 Gy will now be administered to the ovaries of female guinea-pigs, and their oocytes will be collected directly after treatment. They will be cultured according to our standardized techniques and examined for the presence of translocations and/or other chromosome aberrations.

Head of project 4: Prof. Streffer

II. Objectives for the reporting period

1. Impact of gamma-rays (137Cs; dose rate = 0.011 Gy/h = 0.18 mGy/min; total doses = 0.7 and 1.4 Gy) on fetal damage, in particular lethal effects and macroscopically visible malformations, after exposure of oogenesis stages.

Objectives for the next reporting period

1. Impact of gamma-rays (137Cs; dose rate = 0.28 Gy/h = 4.6 mGy/min; total dose = 2.8 Gy) on fetal damage, in particular lethal effects and macroscopically visible malformations, after exposure of spermatogenesis stages.

2. Fertility of female mice half a year after exposure to low dose rate 137Cs gamma-rays.

III. Progress achieved including publications

INTRODUCTION

Previous experiments have shown that one of the mouse strains kept in our Institute (Heiligenberger mice; previously colony-bred, now inbred strain; similar to NMRI-mice) does respond with a higher frequency of malformations after radiation exposure of oogenesis stages. This was true for a high dose rate (1 Gy/min; X-rays) and a low dose rate (0.28 Gy/h = 4.6 mGy/min; 137Cs gamma-rays). Due to the marked radiation sensitivity of mouse oocytes with regard to lethal effects, it was not possible to study the effect of radiation exposure in older oocyte stages ("older" means week 5 and shorter before conception for the high dose rate and week 6 and shorter before conception for the low

dose rate). Therefore, we examined an even lower dose rate, that is 0.011 Gy/h = 0.18 mGy/min.

METHODOLOGY

Female mice were irradiated with gamma-rays (137Cs; dose rate 0.18 mGy/min) or sham-irradiated. (The slightly different dose rates mentioned in the Proposal are due to measurements which turned out to be inaccurate; we repeated the dosimetry of our 137Cs source meanwhile, using various techniques; the dose rates and total doses mentioned in this report are the corrected ones). Mating started immediately after radiation exposure or, in some cases, after a delay of some weeks. Plug control was carried out every morning and those females with a vaginal plug (unequivocal sign of copulation) were singled out. 19 days after copulation (day of copulation = day 1), the mice were killed by cervical dislocation and the uterine content checked for early resorptions, late resorptions, late fetal death, surviving fetuses, and fetuses with macroscopically visible malformations.

RESULTS

The major problem of studies on radiation effects on female germ cells is the pronounced sensitivity of these cells with regard to lethality. Previous experiments have shown that using low dose rates (e.g. 0.28 Gy/h instead of 60 Gy/h) partly overcomes this problem. Meanwhile we examined an even lower dose rate, i.e. 0.011 Gy/h.

As we had already shown that the most mature oocytes (copulation 1-4 weeks after radiation exposure) did respond with an increased frequency of malformed fetuses after radiation exposure (irrespective of the dose rate), we started mating in the fifth week after exposure in the experiments to be presented here.

Fig. 1 summarizes the data on survival. Since the change of a colony-bred to an inbred keeping of the Heiligenberger mice about two years ago, the number of total litter loss (= mice without any implantation site despite the presence of a copulatory plug on day 1) increased from about 10% to about 40% in the controls. Fig. 1 clearly shows that the very low dose rate used in the

experiments indeed allows survival of some less mature oocytes (weeks 5-7), but that starting with week 8 also under these conditions no surviving oocytes were observed.

Fig. 2 presents the results with regard to the teratogenic effects. First of all, the figure shows another consequence of inbreeding our mouse strain: the control level of malformations (almost exclusively gastroschises) raised markedly and the variability increased (colony-bred: about 1%, inbred: between 1 and 4%). The variability, in particular, is a serious problem with regard to the detection of small effects. Therefore, a total dose of 0.7 Gy is not sufficient to show a statistically significant increase in the number of malformed fetuses in the population size examined. After a total dose of 1.4 Gy, however, a marked increase in the number of malformed fetuses is observed for the rather immature oocytes that were exposed.

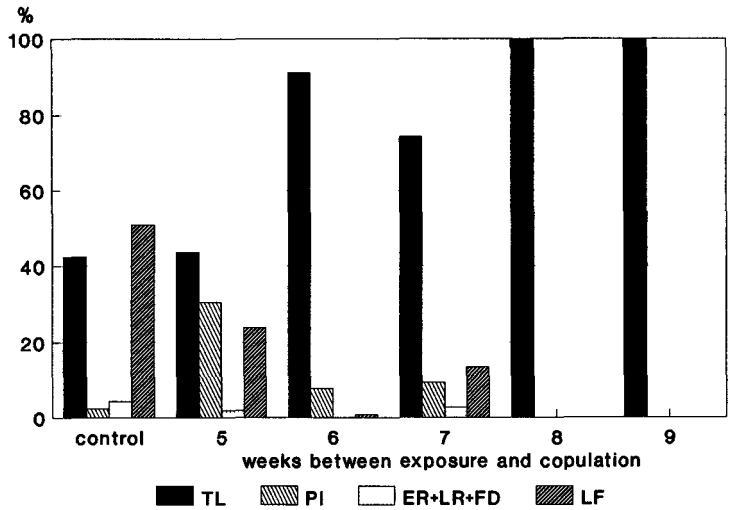


Fig. 1: Lethal effects after a total dose of 1.4 Gy at 0.011 Gy/h (TL=total litter loss; PI=preimplantation death; ER=early resorptions; LR=late resorptions; FD=fetal death; LF=living fetuses)

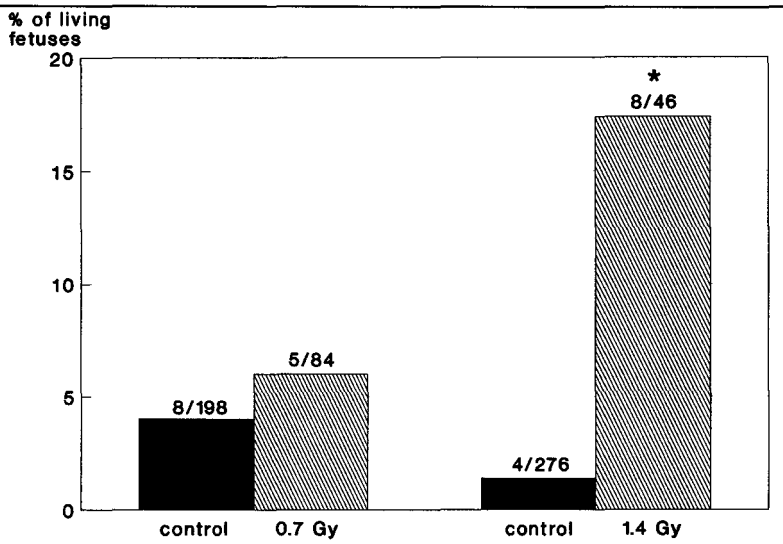


Fig. 2: Malformations after exposure of oocytes to 0.011 Gy/h gamma-rays (time between exposure and copulation: 5-7 weeks; numbers on top of the columns: malformed fetuses/total number of living fetuses; * significantly different at $P<0.01$)

Progress Report

Contract: FI3P-CT920055 **Sector:** B14

Title: Genetic risks associated with exposure to ionizing radiation.

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2)	Van Buul	Univ. Leiden
3)	Cattanach	MRC
4)	de Rooij	Univ. Utrecht
5)	Miró Ametller	Univ. Barcelona - Autónoma
6)	Eeken	Univ. Leiden
7)	Hulten	EBHA

I. Summary of Project Global Objectives and Achievements

An adequate understanding of the genetic risks associated with radiation exposure as well as the mechanisms of mutation induction are essential for an informed action to protect mankind from the harmful effects of radiation. To this end our present project addresses the strategy of risk estimation based on experimental data in animals to determine factors important to the mechanisms of mutation induction by radiation exposure to germ cells. Experiments were conducted to improve risk extrapolation procedures as well as to determine the influences of DNA damage repair and cell killing on the levels of mutation induction by radiation. Genetic and molecular techniques have been employed to determine the regions prone to radiation induced damage and to characateriaze the types of DNA damage resulting in transmitted mutational effects. The major achievements for the reporting period may be summarized as follows.

1) The radiation doubling dose for enzyme activity mutations in spermatogonia of the mouse is estimated to be 1.5 Gy. This observation emphasizes the conclusion that estimations of human genetic risk should be based on animal experimental results for comparable genetic endpoints in man.
2) Based on mouse-hamster germ cell mutagenicity results there does not appear to be differences in the sensitivity to mutation induction of germ cells of these mammals.
3) Strain 101/H is not more resistant to the induction of specific locus mutations by radiation than the standard (101xC3H)F_1 genotype although there is a high level of stem cell killing.
4) Groups of large deletions recovered in radiation experiments have been localized on regions of chromosomes 1, 10, and 14 following irradiation of mouse germ cells have been identified and may indicate regions of the mouse genome sensitive to radiation induced effects. The mutations are maintained and will be molecularly analyzed.

5) Radiation-induced translocation yields in treated mutant Steel and Dominant spotting mice indicate the importance of the proportion of the sensitive stem cell population, cell killing and post-irradiation cell proliferation to the levels of translocations actually observed.
6) The ratio of radiation-resistant to radiation-sensitive stem cell spermatogonia of mice is estimated to be 45:55, which approximates the ratio 50:50 for quiescent to proliferating stem cells.
7) Techniques have been established to score for radiation-induced micronuclei of human spermatozoa (in vitro irradiation) via the human-hamster in vitro fertilization methods. A clear dose response curve has been established and such techniques could be modified to assay for effects of in vivo exposures.
8) Drosophila studies indicate that dominant wing mutations mostly map to known haploid insufficient loci. These results are consistent with the hypothesis that radiation mostly induces deletions which will only result in transmitted dominant mutations if a haploid region of the genome is affected.

Head of project 1: Dr. Ehling

II. Objectives for the reporting period

1) Radiation-induced mutation rates to dominant cataract and enzyme activity deficient alleles in male and female germ cells of the mouse will be compared.

2) Radiation-induced mutation rates to dominant cataract and enzyme activity deficient alleles in male and female germ cells of the hamster will be estimated and allow a species comparison of the sensitivity to mutation induction following radiation exposure.

IIa. Objectives for the next reporting period

1) Mouse-hamster radiation mutagenicity experiments will be c ontinued.
2) Experiments will be initiated to estimate the sensitizing effect of 3-aminobenzamide in mouse spermatogonia on the radiation-induced mutation rate to dominant cataract alleles.
3) Recovered dominant cataract and enzyme activity mutations will be genetically characterized.

III. Progress achieved including publications

1) Experiments to estimate the radiation-induced mutation rates following 2 + 2 Gy (24 h fractionation interval) in germ cells of the mouse as well as an extension of the control group have been initiated. Preliminary results are given in Table 1.

Table 1: Mutation rates in control and 2 + 2 Gy (24 h fractionation interval) irradiated germ cells of the mouse

Group	Dominant Cataract			Enzyme Activity		
	Mutants	Offspring	MR*	Mutants	Offspring	MR*
Control	1	22,594	0.1	1	15,766	0.5
2 + 2 Gy						
post-gonia	0	1,949	-	1	1,098	7.6
gonia	6	18,521	1.1	2	9,370	1.8
oocytes	2	5,081	1.3	6	5,898	8.5

* MR = per locus mutation rate x 10^{-5}. For the dominant cataract results 30 loci are assumed. For the enzyme activity results 12 loci were screened.

The first enzyme activity mutation has been recovered in the control group and allows an initial estimate of the spontaneous mutation rate at these loci. The results are important since with an estimate of the spontaneous mutation rate, the radiation doubling dose for the induction of enzyme activity mutations may be calculated. For exposure of spermatogonia, the radiation doubling dose is estimated to be 1.5 Gy. This value is higher than the radiation doubling dose for recessive specific locus mutations and comparable for the doubling dose calculated for induced dominant cataract mutations. If these preliminary results should be confirmed, they would indicate that extrapolations of animal mutagenicity data to estimate human genetic risk can only be made for comparable genetic endpoints in mouse and man. It should be noted, however, that the dominant cataract and enzyme activity results represent a single spontaneous mutation, which would render high variability to the point estimations of the doubling doses.

A series of mouse germ cell mutagenicity experiments following 2 + 2 Gy (24 h fractionation interval) were initiated. We had originally intended to carry out hamster experiments following 3 + 3 Gy exposure (comparable to mouse results from previous reporting periods). However, the hamster proved to be more sensitive to these higher exposure levels and due to high cell killing were rendered permanently sterile. The mouse results for both dominant cataract and enzyme activity alleles, where sufficient numbers of offspring were screened and mutations were recovered, are consistent with observations for mammalian germ cell mutagenicity experiments for specific locus alleles, i.e. mutation rates in post-spermatogonia and oocytes are similar and higher than the mutation rates observed in irradiated spermatogonia. Further, the per locus mutation rates are higher for the enzyme activity alleles than for the dominant cataract alleles.

2) Comparable experiments to estimate the radiation-induced mutation rates to dominant cataract and enzyme activity alleles following 2 + 2 Gy (24 h fractionation interval) in germ cells of the hamster were initiated. Preliminary results are presented in Table 2.

Table 2: Mutation rates in control and 2 + 2 Gy (24 h fractionation interval) irradiated germ cells of the hamster

Group	Dominant Cataract			Enzyme Activity		
	Mutants	Offspring	MR*	Mutants	Offspring	MR*
Control	0	14,065	-	0	2,807	-
2 + 2 Gy						
post-gonia	0	940	-	0	427	-
gonia	3	8,813	1.1	0	1,195	-
oocytes	2	1,286	5.2	0	2,762	-

* MR = per locus mutation rate x 10^{-5}. For the dominant cataract results 30 loci are assumed. For the enzyme activity results 12 loci were screened.

Results are still very preliminary. The only conclusions which can be made are a) there is no large species difference in the radiation-induced dominant cataract mutation rates between mouse and hamster, and b) for the dominant cataract results in the hamster the observed radiation-induced mutation rate following exposure of oocytes is higher than the mutation rate following exposure of spermatogonia.

Publications

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U.H. Ehling (1991) Genetic risk assessment. Ann. Rev. Genet. 25, 255-280.

J. Kratochvilova and J. Favor (1992) Allelism tests of 15 dominant cataract mutations in mice. Genet. Res., Camb. 59, 199-203.

S. Merkle and W. Pretsch (1992) A glucophosphate isomerase (GPI) null mutation in Mus msuculus: Evidence that anaerobic glycolysis is the predominant energy delivering pathway in early post-implantation embryos. Comp. Biochem. Physiol. 101B, 309-314.

S. Merkle and W. Pretsch (1992) Characterization of two electrophoretic lactate dehydrogenase-A mutants in Mus musculus. Biochem. Genet. 30, 49-59.

S. Merkle, J. Favor, J. Graw, S. Hornhardt and W. Pretsch (1992) Hereditary lactate dehydrogenase A-subunit deficiency as cause of early postimplantation death of homozygotes in Mus musculus. Genetics 131, 413-421.

Head of project 2: Dr. Van Buul

II. Objectives for the reporting period

During the reporting period we completed the dose-response studies for the induction of translocations in steel mouse mutants and their comparison with normal mice (5 and 7 Gy). Construction of the dose-response curve for scid mice was initiated (0.25, 0.50, 0.75, 1, 2, 3 and 4 Gy) and the relation between cell killing and the induction of translocations studied in synchronized spermatogonial stem cells of normal mice. Furthermore the repair of X-ray induced DNA single strand breaks was measured in individual oocytes of the mouse and compared with that in in vitro cultured fibroblasts and human lymphocytes.

III. Progress achieved including publications

Translocations in stem cell spermatogonia

Further studies on the dose-effect relationships for X-ray induced translocations in dominant spotting ($W^V/+$) and steel ($Sl^{con}/Sl/^{con}$) mice with 'primate type' of spermatogenesis confirmed earlier observations (see report 1991) that the recovered yield of translocations was lowered in both mutants with steel being most extreme. Remarkably however, no indications for enhanced cell killing were obtained in the mutants and consequently the peak yields of translocations occurred at the same dose level of 6 Gy as in normal mice. Histological analysis suggested that the post-irradiation recovery of the germinal epithelium was retarded in the mutants with again steel being most extreme. These differences in differentiation-multiplication patterns of regenerating spermatogonia after irradiation are probably responisble for the reduced recovery of translocations from the mutant mice.
Collection of data on cell killing and translocations in a specific stage (G_1) of the spermatogonial stem cell cycle via

combined hydroxyurea (HU), 3-aminobenzamide (3-AB) and X-ray treatment revealed a perfect correlation between these two parameters (collaboration with de Rooij, Utrecht).
Not yet completed dose-response studies in DNA-repair deficient severe combined immunodeficient (scid) mice indicated extreme low induction rates of translocations never increasing above 0.5%.
The results obtained with steel and dominant white spotting mice provided experimental evidence that not only known factors, such as proportions of sensitive and resistant cells and the ration between cell killing and translocation induction, contribute to the final recovery of chromosomal damage from irradiated spermatogonial stem cells, but also the post-irradiation proliferation-differentiation kinetics of the surviving stem cells play an essential role. Correlative studies between cell killing and the induction of translocations indicated a perfect correlation in synchronized cell populations but in heterogeneous situations different results can be obtained.

Induction of DNA breaks in mouse oocytes

Using a modified single cell gel electrophoresis method we have studied the repair of DNA single strand breaks in normal mouse oocytes. The rate of strand breaks is expressed as migration coefficient which is calculated by measuring generated 'comets' with a computerizaed image analysis system coupled with a CCD camera. The repair of breaks in oocytes was much delayed compared to somatic cells such as fibroblasts or lymphocytes (mean repair time 45 min vs. 8 min in somatic cells).

Publications

Buul, P.P.W. van (1992) Biological indicators and radon exposure, Proc. 33rd Dutch Federation Meeting ISBN 90-70248-12-3 (Abstract) p. 57.

Buul, P.P.W. van, C.M.J. Seelen, K. Schöpink and J.H. Goudzwaard (1992) Absense of translocation induction in mouse stem cell spermatogonia by chemical mutagens probably due to selective elimination, Mutagenesis 7, 271-275.

Buul, P.P.W. van, J.H.. Goudzwaard and C.M.J. Seelen (1992) Induction of chromosomal aberrations in mouse premeiotic germ cells following combined treatments with X-rays and chemical clastogens, Int. J. Rad. Biol. 62, 361 (Abstract).

Buul, P.P.W. van, C.M.J. Seelen and J.H. Goudzwaard (1992) Meiotic delay of spermatocytes carying X-ray induced translocations, Cytogenet. Cell Genet. 60, 146-149.

Buul, P.P.W. van, C.M.J. Seelen, K.H. Schöppink and D.G. de Rooij (1992) Induction of chromosomal aberrations in spermatogonial stem cells of steel and dominant white spotting mice by single X-ray exposures, Int. J. Rad. Biol. 62, 376 (Abstract).

Head of project 3: Dr. Cattanach

II. Objectives for the reporting period

1) To complete an investigation of the specific locus mutation response of inbred 101/H mice to 6 Gy spermatogonial X-irradiation.

2) To reinvestigate the translocation dose-response curve of 101/H mice following X-irradiation.

3) To investigate the specific locus mutation response to X-rays of stem cell spermatogonia from standard mice following stem cell depletion by TEM or following HU or 3AB pretreatments.

4) To characterise genetically and cytogenetically chromosome deletions and other gross forms of chromosome changes detected in F_1 animals following paternal X-irradiation.

5) To characterise molecularly a series of mutations at the X-linked *Mo* (Menkes') locus using conventional and pulse field gel electrophoresis.

III. Progress achieved including publications

1) Final results on the 101/H specific locus mutation frequency following a 6 Gy X-ray dose have been obtained. The observed mutation frequency was 23.60×10^{-5} mutations/locus/gamete (10,894 progeny), exceeding rather than being less than the concurrent control (8.88×10^{-5}; 19,297 progeny scored) or the historical control of Russell's (13.62×10^{-5}). Although neither experimental - control difference is statistically significant, there is clearly no indication that the specific locus mutation response of 101/H males to X-rays follows the subnormal translocation responses previously recorded. Prolonged sterile periods confirmed high levels of stem cell killing.

2) To verify that the original 101/H translocation findings were not an artefact caused by the extensive damage to the germinal epithelium the translocation dose-response curve has been reinvestigated using a more refined technique. The results have yet to be fully analysed but appear to confirm the earlier work. Translocation yields were lower than obtained with the standard hybrid control throughout most of

the dose range and the peak yield occurred at a lower dose. Based on duration of sterile periods and recovered testis weights, high levels of stem cell killing were again indicated. There would appear to be a real discord between the specific locus and translocation responses to spermatogonial X-irradiation in the 101/H strain.

3) The combined chemical-X-ray specific locus studies are in progress. Some 8000 F_1 young have been generated so far from 4 different treatment groups. Further data will be required to allow any firm conclusions to be drawn.

4) Large, cytogenetically visible deletions involving 14 of the 19 autosomal chromosomes have now been found and clusters of deletions identified by characteristic phenotypic effects, have been detected on chrs 1, 10 and 14. The breakpoints have been distinguished in all deletions and genetic studies to map those relative to marker genes are in progress. Complementation studies with chr 10 deletions (associated with *Sl* mutations) are also underway. Duplications and balanced or unbalanced forms of translocations associated with phenotypic effects are also being found and these are being mapped wherever possible. Detail of much of the results have been published. Stocks carrying such "mutations" are being stored in the Harwell frozen embryo bank; tissues for DNA studies are also being kept.

5) Considerable progress has been made with the molecular genetic analysis of the genomic region surrounding the X-linked *Mo* locus, the postulated murine homologue of Menkes' disease in man. The genomic organisation of the region has been found to be identical in man and mouse. However, interestingly, no genomic lesions have been detected in a series of 10 spontaneous and X-ray induced *Mo* mutations analysed by conventional and pulsed field gel electrophoresis. A significant additional finding was the discovery that 2 of the 4 mutations that arose in radiation studies did not occur in the irradiated X, ie they were actually of spontaneous origin.

Publications

1. George, A.M., V.R. Reed, C. Rasberry, B.M. Cattanach, M.F. Lyon and Y. Boyd (1992) Molecular analysis of mottled mutations, Mouse Molecular Genetics Conference, Dourdan, France (abstract).

2. Reed, V., A.M. George, S.H. Laval and Y. Boyd (1992) Molecular analysis of the *Ar - Pgk1* - region of the mouse X chromosome, Sixth International Mouse Genome Mapping Workshop, Buffalo, USA (abstract).

3. Reed, V., A.M. George, P. Glenister, J. Chelly, A.P. Monaco, Z. Tumer, N. Horn, B.M. Cattanach, M.F. Lyon and Y. Boyd (1993) Analysis of mutations at mottled, the postulated murine homologue of Menkes' syndrome. 4th X Chromosome Workshop Report, Cytogenetics and Cell Genetics, (in press) (abstract).

4. Reed, V., A.M. George, C. Rasberry, B.M. Cattanach, M.F. Lyon and Y. Boyd (1993) Molecular analysis of a series of mutations at the mottled locus, Mouse Genome, (in press).

5. Cattanach, B.M., M.D. Burtenshaw, C. Rasberry and E.P. Evans (1993) Large deletions and other gross forms of chromosome imbalance compatible with viability and fertility in the mouse, Nature Genetics, 3, 56-61.

6. Cattanach, B.M. and E.P. Evans (1993) Radiation-induced large deletions and other gross forms of chromosome imbalance in the mouse, CEC publication, (in press).

7. Evans. E.P., C.V. Beechey, M.D. Burtenshaw and A.G. Searle (1993) T(In1;5)44H, a complex mouse chromosomal rearrangement with a phenotypic effect, Cytogenetics and Cell Genetics, 63, 66-72.

IV. Objectives of next reporting period

1) To complete the 101/H translocation study.

2) To continue to the specific locus mutation studies with compound chemical X-ray treatments.

3) To initiate a collaborative study with Drs de Rooij and van Buul to elucidate the basis of heterogeneity in radiosensitivity of spermatogonial stem cells.

4) To continue the characterisation of deletions and other categories of chromosome change deriving from recent and ongoing specific locus mutation experiments.

5) To continue with molecular genetic analysis of the *Mo* locus.

Head of project 4: Dr. de Rooij

II. Objectives for the reporting period

The objectives for the reporting period were:
1. To further analyse, write down and publish the findings from the previous contract period.
2. To collect the data of the dose-response experiment for fission neutron irradiation.
3. To start the experiment to determine the radiosensitivity for the induction of reciprocal translocations in stem cells resistant towards cell killing by irradiation and in sensitive stem cells.

III. Progress achieved including publications

1. The final analysis of the data of the dose-response experiment for cell killing by X-irradiation revealed that the sensitive, quiescent spermatogonial stem cells have a D_0 value of 1.4 Gy and the resistant, proliferating stem cells a D_0 of 2.6 Gy. Based on the D_0 for the total population of stem cells of 2.1 Gy, a ratio of 45:55% of sensitive to resistant spermatogonial stem cells could be estimated. This ratio is close to the ratio of 50:50% for quiescent to proliferating stem cells estimated from the spermatogonial cell counts.

Although these data cannot be easily fit into the formula used by Leenhouts and Chadwick, we have tried to combine them with those on translocation induction. The relation describing the translocation induction was assumed to be comparable with the relation for the induction of cell death, i.e. an exponential function of the dose. With a D_0 for cell killing of 1.4 Gy belonging to the fraction of 0.45 of sensitive cells and a D_0 of 2.6 Gy belonging to the fraction of 0.55 of resistant cells, a good fit was obtained when the translocation yield (Y; in % abnormal cells) after a radiation dose (D) was described by $Y=e^{\tau D}$, with $\tau=1$ for the sensitive stem cells and $\tau=0.1$ for the resistant stem cells (Figure 1). The approach of describing the observed translocation induction is the reverse of previous studies, in that we took the cytological data as a starting point instead of the

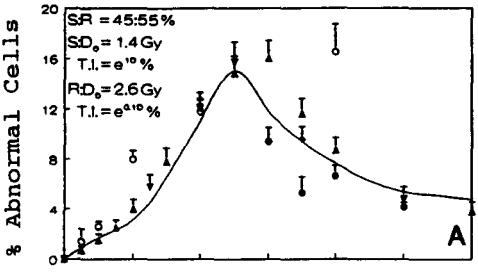


Figure 1. Description of the relation between cell killing and translocation induction after single doses of X-rays. T.I. is the translocation induction in percentage of cells with translocations, with a maximum of 100%.

genetic data. Whether the values for translocation induction assumed here really belong to the sensitive and resistant stem cells has to be further examined. This will be done in the ongoing experiment using stage-synchronized mice by the vitamin A deficiency / vitamin A replacement method.

2. The cell counts involved in the dose-response experiment for 1 MeV fission neutron irradiation are almost finished now. At 10 days after all doses used it was found that the numbers of undifferentiated spermatogonia present were not equally distributed over the stages of the cycle of the seminiferous epithelium (Figure 2). These undifferentiated spermatogonia at

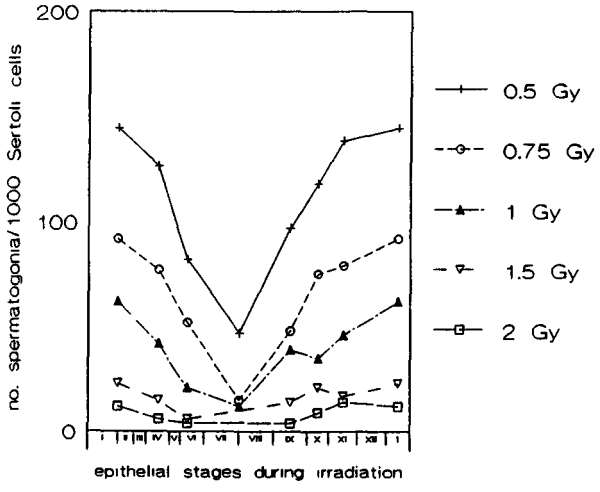


Figure 2. Numbers of undifferentiated spermatogonia per 1000 Sertoli cells at 10 days after 1 MeV fission neutron irradiation in various stages of the epithelial cycle.

10 days after irradiation are derived from surviving spermatogonial stem cells. Consequently, the numbers of undifferentiated spermatogonia found after the various doses can be taken a as a measure of the numbers of surviving stem cells. Dose response curves were generated for various epithelial stages and preliminary D_0 values were derived. In figure 3 these D_0 values have been plotted against the epithelial stage the stem cells were in at the moment of irradiation. The pattern found is very much alike that after X-irradiation, with a low in stages VII-VIII and higher values in stages XI-II. In stages VII-VIII the D_0 value is about 0.33 Gy and in stages XI-II it is about 0.60 Gy. The RBE for fission neutron irradiation can be calculated to be about 4.

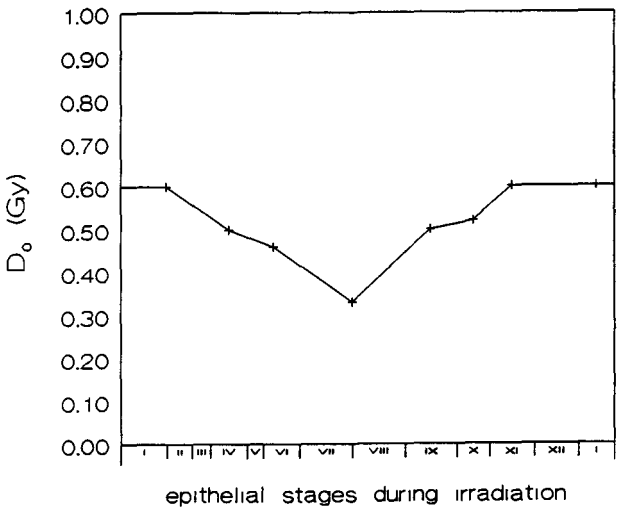


Figure 3. D_0 values for cell killing by 1 MeV fission neutron irradiation of spermatogonial stem cells in 3H1 hybrid mice in various stages of the epithelial cycle.

The next thing to do in this experiment will be to determine the size of the repopulating colonies at 10 days after irradiation in the various stages. Together with the data already obtained it will then be possible to calculate the actual numbers of radioresistant and radiosensitive stem cells per testis in 3H1 mice.

3. Together with Drs. Cattanach, Burtenshaw and Evans at Harwell an experiment is being done to determine the radiosensitivity for the induction of reciprocal translocations in stem cells resistant towards cell killing by irradiation and in sensitive stem cells. Hereto, mice will be irradiated in which the seminiferous epithelium is synchronized by way of the vitamin A / vitamin A replacement method. No results have been obtained as yet.

Publications

1. Van der Meer, Y., R.A.J. Tegelenbosch, B.M. Cattanach, and D.G. de Rooij (1992) Proliferative activity and radiosensitivity of spermatogonial stem cells in C3H/101 F1 hybrid mice. Int. J. Radiat. Biol. 62, 361. Abstract.

2. Van der Meer, Y. (1993) Effects of cytotoxic treatment on mouse spermatogonia. 1993. The relation to proliferative activity. Thesis, Utrecht.

3. Van der Meer, Y., B.M. Cattanach, and D.G. de Rooij The radiosensitivity of spermatogonial stem cells in C3H/101 F1 hybrid mice. Mutation Res. Accepted pending revision.

4. Tegelenbosch, R.A.J., and D.G. de Rooij Quantitative study of spermatogonial kinetics in the C3H/101 F1 hybrid mouse. Mutation Res. Accepted pending revision.

Head of project 5: Dra. Miró Ametller

II. Objectives for the reporting period

The micronucleus test was established in bone marrow erytrocytes and cultures somatic cells as a simpler and faster method to measure chromosomal damage in mutagen-exposed cells. The scoring of micronuclei can also be applied to the human sperm-hamster oocyte fertilization system providing a simple method for assessing radiation-induced chromosomal damage in human spermatozoa. To this end, the proposed objectives for the present period were:

1. The establishement of the technique to analize micronuclei in 2-cell human-hamster embryos.

2. The establishement of criteria to score micronuclei.

3. The selection of an adequate control donor and the irradiation of semen samples provided by this donor.

4. To carry out a parallel dosimetric study by the analysis of radiation-induced chromosome aberrations as a reference to compare the data of radiation-induced micronuclei.

III. Progress achieved including publications

1. Establishement of the micronucleus assay

During the reporting period we have incorporated the micronucleus test into the human sperm-hamster oocyte fertilization system. To score for micronuclei only monospermic eggs can be taken into account, because scoring of micronuclei in embryos from eggs penetrated by more than one spermatozoon would defflect the dose-effect curve. Thus, prior to micronucleus analysis monospermic zygotes have to be identified under an inverted microcope with Nomarski interferential contrast. By culturing these zygotes during 15-17 hours, we have obtained two cell human-hamster embryos that can be observed under the phase contrast microscope to ascertain the presence or absence of micronuclei (Figure 1).
Thus, the micronucleus assay in two cell human-hamster embryos has been completely established in our laboratory.

2. Criteria for identifying micronuclei

In human-hamster embryos, micronulei appear as minute spherical bodies with morphological characteristics identical to the main nucleus of the cell. In preliminary tests we have established some criteria for identifying micronuclei:

- Micronuclei are scored in two cell embryos in which the main nucleus of each blastomere can be visualized.

- The nucleus of the second polar body is so small that it may be mistaken for a micronucleus when the polar body is accidentally overlaid on the blastomere. To avoid confusion, only micronuclei appearing in addition of a visible second polar body are scored (Figure 2).

- Micronuclei usually contain several nucleoli of small size and they are not linked to the main nucleus via a nucleoplasmic bridge.

3. Irradiation of semen samples

A control donor whose spermatozoa early penetrate hamster eggs has been selected. Semen samples of this donor have been splitted in straws containing 0.25 ml of semen each and irradiated at doses of 0.1, 0.25, 0.5, 1, 2, and 4 Gy of ^{60}Co gamma rays. To irradiate straws we have designed an apparatus were straws remain immersed in a water bath at 32.5 ^{0}C, which is the temperature of the human testis.

4. Efficiency of the micronucleus test

To ascertain the efficiency of the micronucleus test, the incidence of micronuclei in 2-cell embryos can be compared with the incidence of structural chromosome aberrations in human spermatozoa. Thus, we carried out a parallel dosimetric study by the analysis of radiation-induced chromosome aberrations in human spermatozoa. Total numbers of 98, 32, 36 and 52 sperm derived complements were karyotyped after doses of 0, 0.25, 0.5 and 1 Gy respectively. The percent of spermatozoa with structural chromosome aberrations were 6.1, 15.6, 25.0 and 30.8 for the different doses (Table 1). The types of structural chromosome aberrations observed more frequently were breaks, deletions and acentric fragments. Among these aberrations, only acentric fragments and fragments derived from breaks are expected to form micronuclei during the first cleavage division. The existence of a correlation between the number of micronuclei per embryo and the number of breaks and fragments per spermatozoa will be examined in the next period.

Table 1. Frequencies of structural aberrations in human sperm chromosomes after in vitro irradiation and unirradiated spermatozoa.

Dose (Gy)	Number of analysed spermatozoa	% of sperm with structural abnormalities
0	98	6.1
0.25	32	15.6
0.50	36	25.0
1	52	30.8

The objectives for the next period are:

1. To ascertain the human or hamster origin of micronuclei by fluorescent in situ hybridization procedures using denatured probes of total human genomic DNA.

2. The establishement of a dose effect curve for radiation-induced micronuclei in 2-cell human-hamster embryos.

3. To evaluate whether scoring of micronuclei is useful for the quantification of chromosomal damage occurring in human spermatozoa by comparison with the dose-effect curve obtained by the analysis of induced structural chromosome aberrations.

Part of the results of the present period are included in an invited lecture to the 9th Annual Meeting of the European Society of Human Reproduction and Embryology held at Thessaloniki June 28-30, and in a communication to the 23rd Annual Meeting of the European Environmental Mutagen Society, that will be held in Barcelona next September.

Egozcue J: Chromosome abnormalities in human sperm (Invited lecture). 9th Annual Meeting of the ESHRE. Thessaloniki, June 28-30, 1993.

Tusell L, Alvárez R, Miró R, Caballín MR, Genescà A, Ribas M, Egozcue J: Micronucleus test to assess chromosomal damage in human spermatozoa. 23rd Annual Meeting of the European Environmental Mutagen Society. Barcelona, September 27- October 2, 1993.

Figure 1. Two-cell embryo with the second polar body.

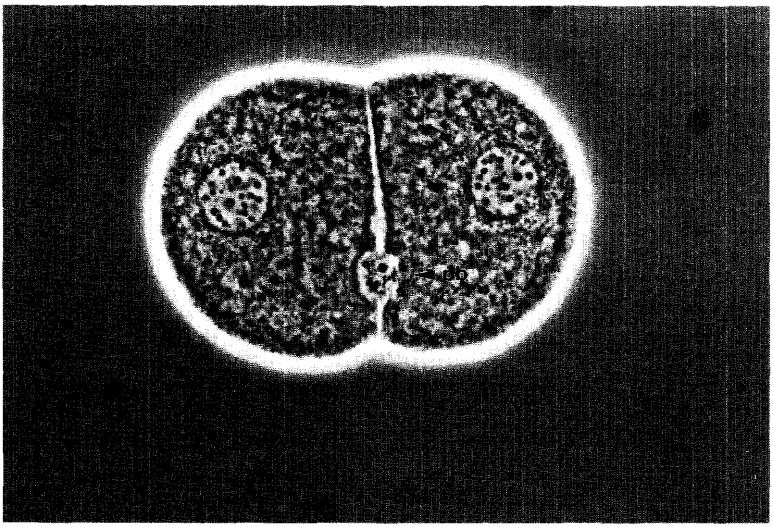
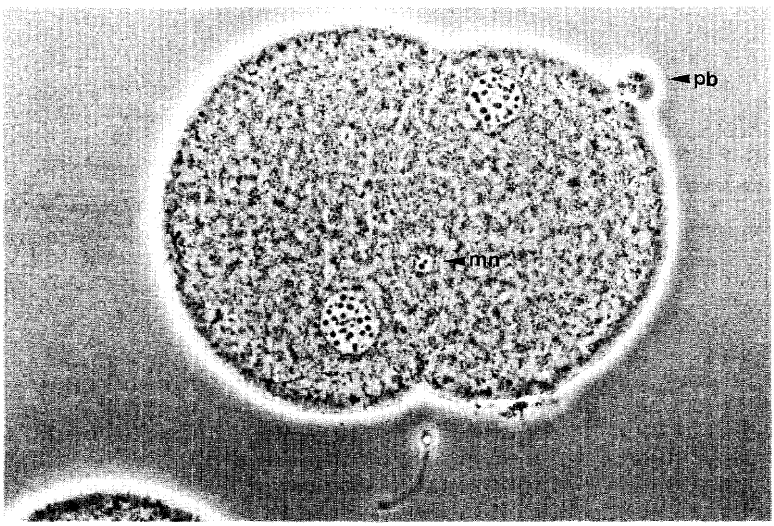


Figure 2. Two-cell embryo with one micronucleus and the second polar body.



Head of project 6: Dr. Eeken

II. Objectives for the reporting period

1. Recovery of X-ray induced recessive and dominant mutations in mature sperm and spermatogonial cells.

Two types of experiments were performed: (1) Irradiated (17.5 Gy) wild type males are mated to M6 females ($In(1)sc^{8}$ + $In(1)dl$-49, y^{31d} sc^{8} w^{a} v f) and irradiated (17.5 Gy) M5 males ($In(1)sc^{S1L}sc^{8R}$ + $In(1)dl49$, w^{a} B) are mated to bi rb cx females. Males are provided with fresh virgins every two/three days, enabling the recovery of successively earlier irradiated stages of spermatogenesis. Recessive visible mutations at the X-linked marker loci y, w, v and f (expt. 1) and at the X-linked marker loci bi rb cx (expt. 2) can be recovered as well dominant visible mutations affecting the morphology of eyes, bristles, legs, halteres and wings.

In the following period 1993 - june 1994, the number of mutations will be further increased especially using experiment type (1). In addition the mutations will be characterized genetically and cytogenetically (pointmutation, multilocus deletion, inversion/ translocation).

III. Progress achieved including publications

In project 6 we investigate the frequency and nature of X-ray induced dominant mutations affecting the morphology and/or viability and livespan in Drosophila melanogaster.

A. Dominant mutations affecting morphology.

The dominant mutations will be viewed in reference to the frequency and nature of recessive visible mutations affecting a number of different X-linked marker genes. Data concerning mutations induced in mature sperm at the white locus have been published (Pastink et al., 1987; 1988; 1990; Eeken et al. 1989, 1991) and manuscripts are being prepared concerning mutations at the v and f loci recovered after irradiation of mature sperm and spermatogonial cells.
A start has been made recovering dominant visible mutations induced by X-rays in experiments comparable to those in which the recessive mutations were found. Sofar we have recovered and brought into stock 13 dominant mutations induced in mature sperm of wild type males, 49 induced in mature sperm of a stock carrying inversions on the X-chromosome (M5) and 9 induced in

spermatogonial cells of wild type males.
The majority of these mutants have morphological wing defects. One of these, resulting in incisions of the margin of the wings, can be due to a mutation in a number of genes (Notch, Beadex, Beadexoid, Beaded). The genetic analysis shows that the majority of mutants of this phenotype are located on the X-chromosome and therefor are either Notch or Beadex (12 induced in mature sperm, 3 in spermatogonial cells). It is known that Notch is a haploid insufficient gene; e.g. null-mutations, including deletions and multilocus deletions, will give rise to this phenotype in females. In males, Notch mutants with wing incisions are lethal. The Notch gene is a complex gene of about 37 kb long that produces several transcripts. The major transcript encodes a 350 kD transmembrane glycoprotein with EGF-like repeat regions. It is expressed throughout development and in addition to its effect on the development of the ectoderm (wings) it plays a role in the development of neurogenic celtypes.
A similar gene as Notch is located on the III-chromosome, Delta. Mutants of this gene give rise to dominant abnormal wing veins. The gene is about 25 kb long and several transcripts are found. The conceptual amino acid sequence indicates again a transmembrane protein with several EGF-like repeats in the extracellular domain. As Notch, Delta is involved in the development and function of neurogenic tissue. This gene is also haplo insufficient e.g. null-mutations, including deletions and multilocus deletions, will give rise to the mutant phenotype. As many as 27 mutants (24 in mature sperm, 3 in spermatogonial cells) have been found showing this phenotype, all located on one of the two autosomes. Most likely the genetic analysis will show that the majority of these are alleles of Delta.
In addition to these two large groups of mutants, 11 have deformed wings as yet of unknown origin, 4 mutants hold their wings at an angle to the body, 2 are Curly like (upturned wings) and 4 have rudimentary wings. In addition to these wing mutants, 3 have been found to express a dominant eyecolor phenotype, two have reduced eyes and one is a bristle mutant. In addition, one X-linked mutant was found with a defect of the thorax.

B. Mutations affecting viability and livespan in heterozygotes.

It is now generally accepted that the major class of X-ray induced mutations are deletions encompassing several genes (multi locus deletions). The phenotype of these mutations is most conveniently described as recessive lethality; e.g. the mutant individual dies when the multilocus deletion is present homozygous. However, it has been shown that these multilocus deletions have additional defects that also affect the individuals carrying such multilocus deletions as heterozygotes (i.e. dominant effects). In Drosophila these effects can be uncovered by measuring for example the relative viability and the relative livespan (Eeken and Sobels, 1990). Genetic analysis of a particular X-chromosome indicates a major factor involved in livespan in the region 5/6 of the polytene chromosome map. It would be extremely interesting to examine multilocus deletions in this region. Suprisingly, it is exactly in this region of the X-chromosome that there is an apparent underrepresentation of multilocus deletions. We set out to generate multilocus deletions in this region. To do this we irradiated M5 males and crossed

them to females with the visible recessive markers bi rb and cx. The polytene chromosome localisation of these genes is 4C5, 4C6 and 5A/B respectively. In 140245 scored chromosomes we found 25 bi, 12 rb and 9 cx mutants. In addition, 11 bi rb double mutants were recovered (not suprising since they are located very close to each other). Around the bi rb genes a number of multilocus deletions were found (10, spanning the region from 4A-5A10). However, around the cx gene only one multilocus deletion was found, extending no further than 5A13. Conclusions from these experiments are: (1) cx is more precisely located: 5A10-5A13 (before (5A1-5C1), (2) apparently there are restrictions that prevent deletions to occur around the cx gene, (3) no such restrictions occur around the bi/rb genes. Possibly the restriction proximal to 5A13 is related to the finding that there may be an important livespan factor in this region.

Publications

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Pastink, A., C. Vreeken, A.P. Schalet and J.C.J. Eeken (1988) DNA sequence analysis of X-ray-induced deletions at the white locus of Drosophila melanogaster, Mutation Res. 207, 23-28.

Pastink, A., C. Vreeken, E.W. Vogel and J.C.J. Eeken (1990) Mutations induced at the white and vermilion loci in Drosophila melanogaster, Mutation Res. 231, 63-71.

Head of project 7: Prof. Hulten

II. Objectives for the reporting period

The main objective for the period has been to develop the fluorescence in situ hybridisation (FISH) technique to be employed for the identification of chromosome aberrations in individual sperm heads. Secondly, we have aimed to organise the collection and establishment of the cell bank to contain samples from the controls and study population of men exposed to ionising radiation following (1) radiotherapy, (2) accidental, (3) occupational and (4) high environmental exposure.

For the next reporting period we aim to start utilising the established FISH technology for a qualitative and quantitative comparison of chromosome aberrations in the controls in relation to the radiation exposed populations. It should be noted that some of these populations have been exposed to relatively low doses of radiation. It is expected therefore that proportionately larger numbers of cell nuclei will have to be analysed to be able to detect a measurable significant difference between unexposed and exposed populations of men. Using whole chromosome paints in the FISH somatic assay we have found there is an indication of a higher involvement of the large heterochromatic blocks such as 1q in comparison to other chromosome segments. We will therefore pay particular attention to the behaviour of these heterochromatic blocks in sperm.

III. Progress achieved including publications

We have utilised repeat sequence DNA probes specific to the X and Y chromosomes as well as autosomes for a study of over 60,000 sperm from unexposed controls (Hultén, M.A., and A.S.H. Goldman (1993) The potential of FISH for meiotic segregation analysis, in: G. Obe and A.T. Natarajan (Eds.), Chromosomal Aberrations. Springer Verlag, In Press; Goldman, A.S.H., Z. Fomina, P.A. Knights, C.J. Hill, A.P. Walker and M.A. Hultén (1993) Analysis of the primary sex ratio, sex chromosome aneuploidy and diploidy in human sperm using dual colour fluorescence in situ hybridisation. Eur. J. Hum. Genet., In Press). The labelling efficiency turned out to be very high with 99.8 per cent of nuclei labelled. We have also used the whole chromosome libraries in combination with repeat sequence DNA probes specific to certain autosomes to investigate the meiotic segregation and gametic output of structural chromosome rearrangements (Goldman, A.S.H., and M.A. Hultén (1993) Analysis of chiasma frequency and first meiotic segregation in a human male reciprocal translocation heterozygote t(1;11)(p36.3;q13.1) using fluorescence in situ hybridisation, Cytogenet. Cell Genet. 63, 16-23; Goldman, A.S.H., and M.A. Hultén (1993) Meiotic analysis by FISH of a human male 46,XY,t(15;20)(q11.2,q11.2) translocation heterozygote: quadrivalent configuration, orientation and first meiotic segregation, Chromosoma 102, 102-111). The results will form the basis for the identification of this type of chromosome aberration in spermatozoa from men exposed to ionising radiation. The cell bank has been organised and the majority of samples to be investigated have been banked.

Contract N° FI3P-CT92-0021

ASSESSMENT, EARLY CELLULAR AND LATE CARCINOGENIC EFFECTS OF EXPOSURE TO RADON AND ITS PROGENY.

Progress report for 1992-1994.

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The main objectives of the initial proposal for 1992-1994 were for the 2 laboratories:

1) to establish standard radon metrology between participant laboratories,
2) to study the effect of exposure conditions on the deposition of radon daughters in the respiratory tract,
3) to continue with the studies on the early subacute effects of radon exposure on epithelial cells of the respiratory tract,
4) to conduct further studies on the early subacute effects of radon exposure on epithelial cells of the respiratory tract using damage to DNA as an index of potential carcinogenicity,
5) to initiate life-time carcinogenicity studies to investigate the effect of dose and dose rate on lung tumour induction,
6) to estimate the doses delivered to the different parts of the respiratory tract.

Significant results have been obtained for each point. Because most studies were performed as collaborative work between the 2 laboratories, a global description of the results is provided.

1) Comparative studies to establish standard radon metrology between participant laboratories have been performed twice in the Razès in 1992 and the Harwell facilities in september 1993. Measurements on the same atmosphere involved influence of pump flow rates, reference standard activities, radon daughter activities, "unattached fractions and total PAEC. After correction for a few parameters, good agreement was found between measurements

performed by the 2 laboratories. Results obtained in Razès have been published (1,2) whereas treatment of those obtained in Harwell is still in progress.

2) Deposition of radon daughters within the respiratory tract has been performed after 214Bi and 214Pb counting in rats exposed for a 3 hour period. Different exposure conditions were studied: in Razès, equilibrium factor 0.8, "unattached" fraction 0.08 and equilibrium factor 0.2, "unattached" fraction 0.70 at PAEC levels from 250 to 2500WL and in Harwell, with "unattached" fraction from 0.01 to 0.53 and PAEC between 40 and 1475 WL. In the Razès experiments, the deposition of radon daughters within lungs was similar for the 2 exposure conditions whereas, deposition in rhinopharynx was about ten times higher for "unattached" fraction at 0.7 than for "unattached" fraction at 0.08.(1-3). Similar results seem to have been obtained in the more complete Harwell study.

3) The early effects of radon exposure on epithelial cells have been characterized by measuring their proliferative indexes as a function of dose and post-exposure time in rats at six months of age. A radiation-induced stimulation of epithelial cell proliferation was observed depending on dose. This stimulation varies depending on the tissue compartment studied: alveoli, bronchiole, bronchi and trachea. For all doses studied, from 120 to 990WLM, a peak for this stimulation occurred 3-4 weeks after exposure which can reach about ten times the control value (4,5). Another experiment has been initiated in june 1993 at Harwell which characterize the proliferative response as a function of the age of the animals from 4 weeks to 40 weeks. Preliminary results show that spontaneous proliferation in rats less than 12 weeks of age is too high to clearly characterize the radiation effects. Further studies are in progress to determine the biological signifiance of this radiation-induced phenomenon.

4) Preliminary studies have been performed on micronuclei induction in tracheal epithelial cells measured in vitro during the primary culture. In vitro experimental approaches are mostly confined to this tissue compartment. Specific micronuclei scoring in post replicative cells can be performed on histological section in all lung compartments after stimulation of cell proliferation by acute exposure to ozone. However, quantitative results can be obtained only by using confocal microscopy (3,6-7). This equipment is now available from december 1993 and methods are being developed for appropriate quantitative analysis.

5) A few carcinogenicity studies have been continued and initiated after exposures in the Razès facility. A Home Office Project Licence to allow exposure of rats and mice in the AEA facility has been applied forward granted for a period of five years. A decision was made to use male Sprague Dawley rats because 1) the low incidence of spontaneous lung tumour in rats, and 2) these are the same strain and sex as used in all previous CEA studies which provided dose-effect relationship for lung tumor induction after exposure to radon. Experiments are in progress to obtain a dose-effect relationship in animals exposed in the Harwell facility.
Different review or studies on the carcinogenic and co-carcinogenic potential of radon have been performed (9-17). Co-carcinogenic studies on the effect of ozone inhalation performed after radon exposure have been initiated. Preliminary results seem to indicate that ozone exposure can have a co-carcinogenic effect (18-19).

6) Dose estimate in the different parts of the respiratory tract has been restricted to the alveolar tissue. This was performed by scoring micronuclei and multinucleated cells in alveolar macrophages extracted by pulmonary lavage. Equivalent doses were determined after homogeneous local lung irradiation by gamma-rays. First results indicate that 1000WLM and 7.5Gy from gamma-rays induce similar nuclear aberration levels. Moreover, the alveolar delivered doses seem to be not dependent on exposure conditions (the 2 conditions described in 2) for the Razès facility), (3,20). Nevertheless, we have shown quite different kinetics of proliferation, micronuclei and multinucleated indexes in alveolar macrophages depending on dose and irradiation type. Moreover, because their renewal rate is not negligible, alveolar macrophages might not be appropriate biological dosimeters. Studies are now developed on epithelial cells in alveoli, bronchiole, bronchi and trachea.

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Progress Report

Contract: FI3P-CT920051 Sector: B15

Title: Induction of osteosarcoma and leukaemia by bone-seeking alpha-emitting radionuclides.

1)	Höfler	GSF
2)	Harrison	NRPB
3)	Wright	MRC
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5)	Skou Pedersen	Univ. Århus
6)	Höfler	GSF
7)	Schoeters	VITO

I. Summary of Project Global Objectives and Achievements

The bone and bone marrow have been shown to be highly sensitive to the carcinogenic effects of alpha-particle emitting radionuclides deposited in bone. It is not yet resolved at what lower limit of radionuclide exposure there is no increased risk of carcinogenesis in exposed subjects. Our participants plan to define the key molecular events that are initiated by irradiation. The long-term objective is to use these changes as molecular indicators of low dose exposure at both individual and population levels. The knowledge obtained from such studies will also serve to complement dosimetric analysis and in vivo dose-effect experiments in determining carcinogenic risk from low-dose exposures. Our experimental approach is two-fold. Firstly, we aim to establish appropriate techniques for molecular analysis of molecular changes within the genome of irradiated target cells., and secondly to identify these radiation-sensitive cell populations within the bone and marrow that are the osteosarcomagenic or leukemogenic targets.

The first year of this project has been characterized by the rapid progress made in establishing a variety of molecular biological techniques for the identification of radiation-induced genomic alterations. Of note is the development of PCR-based techniques to allow the retrospective molecular analysis of archived pathological specimens, accumulated over many years of in vivo radiation carcinogenesis. A catalogue of genomic alterations in radiation-induced murine osteosarcoma is being compiled, to date alterations in the tumor suppressor gene p53 and amplification of myc oncogene appear characteristic. In addition considerable progress has been made in confirming the role of endogenous retrotransposable elements in contributing to the genetic instability following irradiation. Continued analysis of the regulation of retroviral expression has revealed that normal cellular pathways controlling gene transcription may interact with retroviral sequences directly to mediate retrotransposition events. In a most dramatic development a method has been established to detect alterations in the number of retroviral integration sites in tumors.

Of equal importance have been the efforts made in vivo and in vitro to identify the marrow stem cell and osteoprogenitor populations which are sensitive to alpha-particle induced genetic damage. It has now been established that murine haematopoietic marrow stem cells surviving alpha-irradiation may proliferate and seed tissues with cell populations containing alpha-particle induced chromosomal aberrations. A radiation-sensitive osteogenic stem cell population has been identified in marrow, and the response to radiation is being dissected.

Animal studies will continued to play a major role in our program. In this portion of the project a transgenic mouse breeding program has been established to provide a range of animal and cell lines harboring genetic mutations which may impinge upon the pathways of radiation-induced carcinogenesis. The role of genetic background in determining radiation sensitivity has been further investigated by experiments showing that transmissible germ-line mutations play an important role in experimental multigenerational carcinogenesis. In vivo studies to determine the correlation between radionuclide distribution, dose and the sensitivity of specific marrow and bone populations have now been concluded and are entering the analytical phase. These studies have shown that the distribution and type of alpha-emitting radionuclide are important determinants of carcinogenic effect excerted upon the sensitive populations in marrow and bone.

Head of project 1: Dr. Höfler

II. Objectives for the reporting period

The molecular events responsible for radiation-induced carcinogenesis have proven amenable to molecular biological analysis. The characterization of radiation-induced alterations in gene regulation and expression will increase our understanding of the mechanism of radiation-induced carcinogenesis. Ultimately this knowledge will allow a more accurate assessment of the dose-effect relationship, and will permit a monitoring of at risk individuals. Our experimental goal is to use a range of molecular techniques to identify those molecular mechanisms involved in radiation-induced tumorigenesis, with particular emphasis on multigenerational transmission of germ line mutations..

III. Progress achieved including publications

Genetic events

We have developed two methods for identifying genetic alterations in radiation-induced osteosarcomas. We have established a subtractive hybridization strategy to enrich a cDNA library for sequences deleted or inactivated in a particular cell population. The goal being to identify those key genes inactivated by alpha-particle irradiation of the sensitive osteogenic cells whose loss initiates osteosarcoma formation. In our model system we were able to enrich a cDNA library for sequences that were expressed in a well-differentiated rat osteosarcoma cell clone, but which are absent from a poorly-differentiated clone established from the same source. Screening of this subtractive cDNA library allowed us to identify several genes whose expression was down-regulated in the undifferentiated cells. In the second strategy we have applied a polymerase chain reaction-based technique using arbitrary primers (AP-PCR) to amplify random genomic sequences. This technique allows the rapid detection of genomic alterations in tumor cell populations, and will be used to identify potential genetic events in radiation-induced osteosarcoma cells. An additional application of the technique has been developed allowing the mapping of repetitive DNA elements within the genome. Using retroviral integration sites as a target we have been able to demonstrate alterations in the genomic distribution of these sites in transformed cells. In the next

phase of the project we will concentrate our efforts on identifying, in collaboration with partner IMV and VITO, suitable radiation-induced and normal osteoblast cell populations for the subtractive procedure.

Multigenerational carcinogenesis

A series of genetic diseases are recognized in which mutations to key genetic loci are passed from parent to offspring through the germ line and predispose affected offspring to cancers. In addition to these familial cancer syndromes de novo mutations in the parental germ line may be induced by environmental factors. These transmissible mutations may be responsible for a significant proportion of neoplastic disease, and in affected individuals greatly influence their individual risk of carcinogenesis from ionizing radiation.

We have developed an animal model of multigenerational carcinogenesis in which parental exposure to chemical carcinogen (Ethylnitrosourea-ENU) was used to induce germ-line mutations in the mouse. The effect of these germ line events upon a subsequent exposure of the offspring to the alpha-emitting radionuclide 227Th has been analyzed.

Male parents of the murine 102 x C3H stock were treated with a mutagenic dose of ENU and crossed with T-stock females. A proportion of the F1 offspring were exposed to ^{227}Th to determine the influence of paternal effects upon radiation risk. The dose of 227Th was selected to give an approximately 20% incidence of osteosarcoma formation, and at the time of this report this level of incidence has been reached. The data to date indicate that parental exposure to ENU does not increase the incidence of osteosarcoma (see table); rather the rate of osteosarcoma formation is accelerated, with a more rapid appearence of osteosarcomas in the offspring of treated fathers evident between 400 and 600 days post irradiation. The kinetics of the tumor incidence is entirely consistent with the Knudson model of multi-hit carcinogenesis, suggesting that the offspring of ENU-treated fathers have already acquired one or more genetic alterations prior to exposure to radiation. Material from these studies has been prepared for molecular analysis in conjunction with partner TUM.

Cumulative osteosarcoma incidence (corrected for competing risk) after incorporation of 36 kBq/kg ^{227}Thorium (at age of 14 weeks)
Female mice, daughters from ENU-treated or untreated fathers.
(data source: evaluation May 27, 1993)

Time after incorporation (days)	Father treated with ENU N = 66	Father untreated N = 66
201-300	3%	0%
301-400	5%	2%
401-500	11%	3%
501-600	18%	5%
601-700	26%	24%

Publication:

Siggelkow H., Kaesler S., Lanske B., Atkinson M.J. Isolation of osteoblast specific markers using differential hybridization. Osteologie 1 207-211 1982

Head of project 2: Dr. Harrison

II. Objectives for the reporting period

To continue with studies of the distribution of ^{239}Pu, ^{241}Am and ^{233}U within the skeleton of mice, and of their comparative toxicity, with the objective of relating differences in the distribution of dose within the skeleton, and the extent of irradiation of the different cell types, with the observed incidence and distribution of osteosarcoma and myeloid leukaemia.

To undertake studies to determine the distribution and retention of ^{210}Po in the skeleton of rats and marmosets after administration of either of ^{210}Po or ^{210}Pb with the objective of assessing α-doses and leukaemic risk from these naturally-occurring nuclides.

III. Progress achieved including publications

On the basis of the average bone doses calculated from distribution studies, it was estimated that the ratios of administered activity of ^{239}Pu: ^{241}Am: ^{233}U to give the same doses to compare their effects would be 1: 1.05: 5.25. Three activity levels of ^{239}Pu were used, 5, 15 and 25 kBq kg^{-1}, with the expectation, based on the work of Humphreys and colleagues, that the incidence of osteosarcoma in these groups would be about 7%, 15% and 30% respectively. The corresponding activities of ^{241}Am and ^{233}U were 6, 17, and 29 kBq kg^{-1} and 40, 120 and 200 kBq kg^{-1}, respectively. To avoid chemical damage to the kidneys from U, the administration of activity was fractionated over a 3 week period. Groups of mice (50 - 100) were therefore given 9 intraperitoneal injection of the radionuclides in citrate over a 3 month period and a control group was similarly injected with inactive solution.

All the animals in this study have now died, the incidence of osteosarcoma and leukaemia for the different radionuclides are given in the table. A total of 32 bone tumours have been identified to date by X-ray examination of the skeleton at death. Confirmation by histological examination is in progress and further suspected tumours are being evaluated histologically. In animals given ^{239}Pu, the bone tumour incidences are 4, 7 and 16% for the low, medium and high groups respectively. The incidences for ^{241}Am are 1, 4 and 8% and for ^{233}U are 3, 5 and 4% (low, medium and high groups). The incidence of identified bone tumours in mice given ^{239}Pu, expressed in terms of the total time since administration for animals in each group (incidence per 10^5 days), is currently 5.5, 10 and 25 for the low, medium and high groups, respectively. For ^{241}Am, the corresponding incidences are 1.5, 6 and 12, respectively, and for ^{233}U, 4, 7 and 6, respectively (see table).

The myeloid leukaemias are confirmed by blood counts, analysis of blood smears for abnormal cells and by passaging the spleen homogenate into untreated mice to look for myeloid leukaemia development. Under CEC Contract B170037 a number of the leukaemias have been karyotyped and have shown chromosome 2 rearrangements characteristic of acute myeloid leukaemia. Cells from future suspected leukaemias will be karyotyped in the same way.

Twenty-four myeloid leukaemias have been confirmed to date and evaluation of several additional suspected myeloid leukaemias is currently underway. In animals given ^{239}Pu the incidences are 3, 5 and 2% for the low, medium and high groups respectively. The incidences for ^{231}Am are 3, 3 and 0% and for ^{233}U are 5, 8 and 4% (low, medium and high groups). The incidence of identified myeloid leukaemias in mice given ^{239}Pu, expressed in terms of the total time since administration for animals in each group (incidence per 10^5 days), is currently 4, 8 and 3 for the low, medium and high groups, respectively. For ^{241}Am, the corresponding incidences are 4.5, 4 and 0, respectively, and for ^{233}U, 7, 12 and 6, respectively (see table). The results to date suggest a greater incidence of myeloid leukaemia in mice exposed to ^{233}U than in those exposed to ^{239}Pu and ^{241}Am.

Uncertainties in doses to the red bone marrow and leukaemic risk from the natural alpha-emitters ^{210}Po as the daughter of ^{222}Ra and ^{210}Pb have recently been highlighted. Contributions of dose to red bone marrow from ^{210}Po can arise from two different sources. Polonium-210 can enter the circulation after inhalation or ingestion of ^{210}Po or as a result of decay of skeletal stores of ^{210}Pb. Rats were, therefore initially given injections of ^{210}Po citrate to determine its distribution and retention and subsequently rats were given intravenous injections of ^{210}Pb citrate in the absence of its daughters ^{210}Bi and ^{210}Po.

About 10% of injected ^{210}Po was deposited in the skeleton and autoradiographs showed this to be evenly distributed throughout the bone marrow. The figure for skeletal retention fell to 1.5% over 200 days, representing about 12% of the total body burden at this stage; autoradiography showed that this remained at the original site of deposition within the bone marrow. There was an initial rapid loss of about 50% of the activity over 20 days and the remainder was retained with a longer biological half-time of about 140 days. Results are not yet available for the distribution and retention of ^{210}Po after the decay of ^{210}Pb in the body.

Table. Incidence of osteosarcoma and myeloid leukaemia in CBA/H mice following the administration of ^{239}Pu, ^{241}Am and ^{233}U.

^{239}Pu				
Dose kBq kg^{-1}	0	5	15	25
Average bone dose at 500 days (Gy)	0	0.1	0.3	0.5
No of mice entered	100	100	60	50
No of mice dead	100	100	60	50
Myeloid leukaemia	0	3	3	1
Osteosarcoma	0	4	4	7
Mouse days exposure	63321	71710	40127	32271
10^5 x Myeloid leukaemia $days^{-1}$	0	4.2	7.5	3.1
10^5 x Osteosarcoma $days^{-1}$	0	5.6	10.0	24.8
^{241}Am				
Dose kBq kg^{-1}	0	6	17	29
Average bone dose at 500 days (Gy)	0	0.1	0.3	0.5
No of mice entered	100	100	75	50
No of mice dead	100	100	75	50
Myeloid leukaemia	0	3	2	0
Osteosarcoma	0	1	3	4
Mouse days exposure	63321	68959	49558	34665
10^5 x Myeloid leukaemia $days^{-1}$	0	4.4	4.0	0
10^5 x Osteosarcoma $days^{-1}$	0	1.5	6.1	11.5
^{233}U				
Dose kBq kg^{-1}	0	40	120	200
Average bone dose at 500 days (Gy)	0	0.1	0.3	0.5
No of mice entered	100	100	60	50
No of mice dead	100	100	60	50
Myeloid leukaemia	0	5	5	2
Osteosarcoma	0	3	3	2
Mouse days exposure	63321	69761	41933	32720
10^5 x Myeloid leukaemia $days^{-1}$	0	7.2	11.9	6.1
10^5 x Osteosarcoma $days^{-1}$	0	4.3	7.2	6.1

Head of project 3: Dr. Wright

II. Objectives for the reporting period

Previously, we have reported a high frequency of non-clonal chromatid and chromosome aberrations in colonies of cells derived from murine haemopoietic stem cells surviving low doses of alpha-particle irradiation. Our objective in the current reporting period has been to to extend these studies of radiation-induced genetic instability:-

1. To investigate further the probability of murine haemopoietic stem cells surviving a single alpha-particle track.
2. To investigate further the capacity of surviving murine haemopoietic stem cells to proliferate and express alpha-particle induced genetic damage *in vitro* using cytogenetic technique and to extend the studies to mouse strains other than CBA/H.
3. To extend the cytogenetic study of the proliferating progeny of stem cells surviving alpha-particle-irradiation to the *in vivo* situation using bone marrow transplantation techniques.
4. To develop techniques that will allow comparable studies of human bone marrow to be initiated.

III. Progress achieved including publications

1. The probability of murine stem cells surviving small numbers of alpha-particle tracks.

In collaboration with Dr Dudley Goodhead (MRC Radiobiology Unit), inactivation of murine haemopoietic stem cells, assayed as spleen colony-forming units (CFU-S), has been determined after *in vitro* irradiation under well defined conditions with small numbers (≤ 3) of alpha-particles of incident energy 3.3 MeV (LET 121 keV μm^{-1}). The CFU-S compartment is age-structured with the earlier members (day 12 CFU-S) having greater self-replicative capacity than the later members. Later members measured as day 8 CFU-S are multipotential cells with negligible self-replicative capacity and are highly responsive to proliferation and differentiation stimuli.

Exponential survival curves were obtained with inactivation dose coefficients 1.833 ± 0.11 and 1.633 ± 1.22 mm^2 for day 8 CFU-S and day 12 CFU-S respectively, corresponding to inactivation cross sections of 35.6 ± 2.1 and 31.7 ± 1.2 mm^2. The results indicate that these radiosensitive stem cells have a probability of surviving the passage of a single alpha-particle track, estimated at 8 and 18% per particle passage, respectively, if the cells have a diameter of 7 μm (the modal diameter determined by velocity sedimentation studies).

Lorimore, S.A., Goodhead, D.T. & **Wright, E.G.**
Inactivation of haemopoietic stem cell by slow alpha-particles.
International Journal of Radiation Biology, **63**, 655-660, (1993)

2. Preliminary investigation of a second strain of mouse (B6D2F1)

Our initial studies were carried out using bone marrow obtained from the CBA/H strain of laboratory mouse. More recent studies of a second mouse strain have confirmed the induction of chromosomal instability but in these experiments there were fewer colonies with aberrations and fewer abnormal cells within affected colonies (see Table 1). This raises the possibility of an important genetic component to quantitative aspects of radiation-induced genetic instability. We propose to investigate further. the effects of the genetic characteristics of the target cell.

Table 1. Chromosomal instability in CFU-A colonies

Radiation Exposure (Gy)	Colonies with Aberrations	Metaphases with Aberrations	Aberrations/ Metaphase 1 2 3 4	Total Aberrations Chromosome	Chromatid
CBA/H					
0	7/59	7/432	7 0 0 0	0	7
0.5	6/12	19/92	13 3 2 0	8	14
B6D2F1					
0	1/15	1/251	1 0 0 0	0	1
0.5	4/24	10/472	6 2 1 1	4	13

This preliminary study of bone marrow cells obtained from B6D2F1 mice was carried out as described in Kadhim *et al* (1992) *Nature* **255** 738-740. The CBA/H data shown for comparison is taken from this publication.

3. Evidence for long-lived chromosome instability in mice transplanted with bone marrow exposed to alpha-particle irradiation.

In this pilot study carried out to determine whether chromosome instability is transmissible *in vivo*, bone marrow cells were taken from two mice taken at random six months post-transplantation of alpha-particle irradiated (0.5 Gy) marrow. The cells were transferred into culture medium to maintain their viability and 48 hours later examined for cytogenetic aberrations: in each case only 100 male metaphases were examined (the transplant recipient were female). In the first sample, 7 abnormal cells were observed; 6 cells had single abnormalities - 3 chromatid breaks, 2 centric associations, 1 extra chromosome (41XY) and one cell had two aberrations on different chromosomes -a chromatid and a chromosome break. In the second sample, 6 abnormal cells were observed; 5 with single abberations - 2 chromatid breaks, 2 chromosome breaks, 1 hyperdiploid and one cell had two aberrations on different chromosomes - a chromatid and a chromosome break.

These preliminary data are consistent with long-lived, ongoing instability in mice whose haemopoietic system has been repopulated by transplantation of alpha-particle irradiated stem cells. More detailed studies are now in progress.

4. Preliminary investigations to determine whether chromosome instability can be induced in clonogenic cells in human bone marrow by alpha-particle irradiation.

Collaborative studies have led to the development of a quantitative clonogenic assay for human marrow cells comparable to the stem cell assay for murine cells and operationally defined as a CFU-A assay.

> Holyoake, T.L., Freshney, M.G., Konwalinka, G.,, Haun, M., Petzer, A., Fitzsimmons, E., Lucie, N.P., **Wright, E.G.** & Pragnell, I.B. Mixed colony formation in vitro by the heterogeneous compartment of multipotential progenitors in human bone marrow. *Leukemia*, **7**, 207-213, (1993).

This development has allowed us to carry out preliminary studies of the effects of alpha-particle irradiation on human cells comparable to those with murine stem cells.

Preliminary radiation experiments have been conducted with normal human marrow from four donors (see Table 2). The preliminary findings are that in colonies obtained from two of the four donors no chromosome instability was demonstrable. In two samples, however, results qualitatively similar to those obtained with murine cells were obtained but the incidence of affected colonies and affected cells within colonies was lower than with CBA/H marrow. We interpret our preliminary findings as evidence for alpha-particle induced transmissible instability in human stem cells with marked inter-individual variation. The variation may reflect important genetic differences .

Table 2. Non-clonal aberrations in human CFU-A colonies

Radiation Exposure α-particles (Gy)	Colonies with Aberrations	Metaphases with Aberrations
HBM-1		
0	1 / 7	1 / 49
0.25	0 / 5	0 / 37
0.5	0 / 6	0 / 42
HBM-2		
0	0 / 15	0 / 105
0.25	3 / 18	10 / 104
0.5	2 / 4	5 / 20
HBM-3		
0	0 / 3	0 / 21
0.25	0 / 2	0 / 15
0.5	0 / 3	0 / 18
HBM-4		
0	0 / 3	0 / 17
0.25	2 / 5	6 / 32
0.5	1 / 3	4 / 37

Head of project 3: Prof. Dr. V.Erfle
Radiation-induced osteosarcomagenesis in vitro: effects of low dose alpha-irradiation and radiation-activated retroviruses on differentiation pathways

Radiation-induction of osteosarcomas in mice -a model system for radiation carcinogenesis in man- is associated with a dose-dependent activation of endogenous retroviruses. These viruses induce skeletal tumours in the mouse, indicating that

-provirus activation and the expression of infectious retroviruses represents a critical step in radiation osteosarcomagenesis, and

-activation of endogenous retroviral genes may serve as an early molecular marker for osteogenic cells which have been affected by irradiation.

Radiation-activated retroviruses induce bone lesions after a latent period of several months. Hence, additional genetic or epigenetic events appear to be also involved in radiation osteosarcomagenesis. These include

-alterations of the p53 gene,
-enhanced expression of the p53 gene, and
-amplifications of the c-myc gene.

These features which are frequently observed in radiation-induced osteosarcomas.

Transgenic mice, carrying one or more of these mutated or activated genes as a genetic trait develop bone tumours with low incidence. These mice harbour cells in an advanced stage of neoplastic transformation and with increased sensitivity to the transforming activity of additional carcinogenic factors. Retrovirus infection for instance increases bone tumour incidence in both *fos*-transgenics in *myc* transgenics. Cell or tissue cultures established from transgenic mice may therefore serve as an ideal model system for the analysis of the cooperation of α-irradiation, radiation-activated retroviral genes and oncogenes in carcinogenesis.

The objectives of our group are to study the role of radiation-activated retroviral genes in the initiation, fixation, enhancement and/or accelaration of radiation-induced genomic alterations with special emphasis on:

-cooperating effects of α-irradiation and infection with radiation-activated retroviruses on differentiation and neoplastic transformation of skeletal cells, and

-early effects of radiation-induced provirus activation, retrovirus infection and new integration in osteogenic target cells.

Affected cells and tissues will be analysed for the expression of protooncogenes, structural genes typifying osteogenic differentiation, and genes associated with cell proliferation.
As in vitro systems we will include cell and tissue cultures from bone-forming cells established from c-fos, c-myc, c-fosxc-myc hybrids, p53 transgenic and p53-null mice. These strains have been successfully bred in our laboratory. The cells will be irradiated with doses of ^{223}Ra which are known to activate endogenous proviruses and to induce cell transformation (7.4-7400 Bq/ml ^{223}Ra/ml culture medium). In addition, irradiation will be preceeded or followed by infection with AKV MLV, a molecularly cloned prototype of endogenous mouse retroviruses .

Progress achieved including publications

In vitro experiments have been compleeted for the analysis of the effect of ^{224}Ra on osteogenic tissue. The data indicate an enhancement of proliferation together with increased osteogenic differentiation in irradiated tissues (Schmidt et al. Rad Eviron Biophys.,.in press).

Molecular analyses of radiation-induced osteosarcomas showed an inverse corralation of p53 expression and osteogenic differentiation of the tumors (Strauss et al. in press).

A cell culture model for bone progenitor cells has been set up and clonal cell lines have been established from normal mice and from myc-transgenic as well as from myc/p53 transgenic mice.

In vivo experiments are in progress to investigate the effect of ^{227}Th irradiation on p53 transgenic mice. These mice show an increased risk for tumorigenesis including development of osteosarcomas.

Objectives for the next reporting period

-Evaluation of the in vivo effects of ionizing irradiation in p53 transgenic mice

-Establishment of a cell culture system with bone precursor cells from p53 transgenic and p53-null mice, e.g. a cell line with aberrant p53 expression or lacking a functional p53 gene.

-Irradiation of oncogen-transgenic or p53 deficient cell lines with ^{223} Ra, in the presence or absence of retrovirus infection.

Publications

Strauss, P.G., Schmidt, J., Luz, A., Erfle, V. Radiation-induced osteosarcoma: a model for evaluation of molecular pathway to transformation. 1993 Proceedings of the osteosarcoma conference, Pittsburgh, in press.

Strauss, P.G., Mitreiter, K., Zitzelsberger, H., Luz, A., Schmidt, J., Erfle, V. Höfler, H., Inverse correlation of p53 and osteocalcin RNA expression in murine osteosarcomas. 1993 Proceedings of the osteosarcoma conference, Pittsburgh, in press.

Schmidt, J., Linzner, U., Luz, A., Heermeier, K., Silbermann, M., Livne, E., Erfle, V. Irradiation of in-vitro differentiating skeletal tissue with osteosarcomagenic doses of 224Radium: effects on proliferation and osteogenic differentiation. 1993 CEC Proceedings, in press.

Strauss, P.G., Mitreiter, K., Luz, A., Atkinson, M.J., Höflier, H., Schmidt, J., Erfle, V. Mutation of the p53 tumor suppressor in radiaton-induced murine osteosarcomas. CEC Proceedings, in press.

J. Schmidt, K. Heermeier, U. Linzner, A. Luz, M. Silbermann, E. Livne and V. Erfle Osteosarcomagenic doses of ^{224}Radium (^{224}Ra) and infectious endogenous retroviruses enhance proliferation and osteogenic differentiation of in vitro-differentiating skeletal tissue. Rad. Environ. Biophys. in press.

Head of project 5: Prof. Skou Pedersen

II. Objectives for the reporting period

Retroviral proviral elements may be carried in the germ line of birds and mammals and their expression may be repressed in somatic cells although the proviral transcriptional unit is fully functional. We use endogenous proviruses as models for the analysis of factors affecting the activation of retrotransposon elements and for the possible pathogenic effects resulting from the amplification of activated elements.

Objectives for the present and the following reporting period.

1) To analyse molecular mechanisms of radiation-activation of silent proviral genes.

2) To determine pathogenic sequences in proviral genes.

3) To determine proviral sequences conferring tumour type specificity.

III. Progress achieved including publications

1) Activation of silent proviral genes

The expression of endogenous ecotropic proviruses has been shown to vary considerably among inbred strains of mice. This variation, of importance for strain-dependent patterns of disease development, may be determined by *cis*-acting DNA elements within the proviral sequences as well as at the site of integration. The germ line of the low leukaemic BALB/c strain carries, at the locus *emv*-1, a single ecotropic provirus that is never expressed at high levels *in vivo* and rarely spontaneously activated in derived cell cultures. As a model for activation of retrotransposon elements we focus on mechanisms of activation of the *emv*-1 provirus in BALB 3T3 cell cultures.

In initial experiments, actively dividing cultures of BALB 3T3 cells were exposed to a low dose of γ-irradiation (700 rads). Five days after the treatment, cells with an activated provirus were identified by immunostaining using antibodies directed against the proviral Gag or Env proteins. γ-irradiation, which efficiently results in strand breaks, yields low frequencies of virus induction (10^{-3} - 10^{-4}).

Silencing of a provirus may result from the presence of an inhibitor or the absence of specific activating factors. A negative influence may come from DNA-methylation that has a role in the establishment of inert chromatin structures. Irradiation may reduce DNA-methylation as a result of specific damage of DNA at the locus or by a general effect on DNA synthesis or repair. Specific activating factors that may overcome the repression may appear in the cell as a result of biochemical changes after irradiation. Candidates for such factors may be the signal transducer NF-κB or the protooncogene product Fos.

In the present reporting period we have focused mainly on negative effects associated with methylation of DNA at the locus in the untreated cell. As previously seen for other proviruses we observed an increase in frequencies of activation after irradiation in cell cultures treated with BrdUrd during DNA repair. We also found an increase of provirus activation after 5-azacytidine treatment. It suggests that a modification by methylation of DNA double helix at particular sequences can influence endogenous provirus induction. The expression of the provirus may still be sensitive to the influences of chromatin structure, DNA modification and

cis-acting control elements in the flanking host cell sequences.

We have used 5-azacytidine treatment of cells in culture to demethylate a large part of CpG dinucleotides. Southern blotting analysis shows that the region corresponding to the endogenous provirus sequence is highly methylated. A few specific sites in this sequence were found to be only partially methylated in a population of untreated cells. By treatment with 5-azacytidine we have been able to activate proviruses in about 20% of the cells. In nuclear expracts we have detected proteins that bind to a methylated DNA-probe from the provirus promoter. A search for such proteins may be important to understand the mechanisms of repression and activation of provirus transcription.

2) Pathogenicity of proviral genes

The proviral LTR may regulate both retroviral and adjacent genes. Provirus reinsertions resulting from activation of an endogenous retrovirus may therefore be part of a cascade of radiation-induced events leading to transformation. We have developed a hemi-specific polymerase chain reaction technique that allows the determination of DNA-sequences adjacent to a provirus by a simple two step procedure (Sørensen et al., J. Virol. in press). We are currently using this procedure to map provirus integrations in tumor DNAs and study fundamental aspects of transcriptional interferences between a provirus and its flanking DNA in cell culture models. These model studies employ LTR regions from strongly as well as weakly pathogenic viruses.

3) Proviral sequences conferring tumour type specificity

From our previous work we have available a panel of molecular clones of endogenous proviruses or proviruses from radiation induced or non-radiation induced osteosarcomas and tumours of the haematopoietic system. These proviruses show a variety of phenotypes with respect to pathogenicity as summarized in the final report for contract Bi7-002. In a large series of long-term experiments we look at the pathogenic potency and tumorigenic specificity of specifically engineered mutated and recombinant viruses. Tumour DNAs are analyzed for proviral integration sites and for alterations in LTR sequences that occur during tumorigenesis.

All studies are carried out in close colaboration with Institut für Molekulare Virologie, GSF-München (FRG).

Publications and manuscripts:

Sørensen, M.S., Duch, M., Paludan, K., Jørgensen, P., and Pedersen, F.S.: Measurement of hygromycin B phosphotransferase activity in mammalian cell extracts by a simple dot-assay. Gene 112, 257-260 (1992).

Pedersen, K., Lovmand, S., Jørgensen, E.C.B., Pedersen, F.S., and Jørgensen, P.: Efficient expression and replication of murine leukemia virus with major deletions in the enhancer region of U3. Virology 187, 821-824 (1992).

Jørgensen, E.C.B., Pedersen, F.S., and Jørgensen, P.: Matrix protein of Akv murine leukemia virus: Genetic mapping of regions essential for particle formation. J. Virol. 66, 4479-4487 (1992).

Nielsen, A.L., Pallisgaard, N., Pedersen, F.S., and Jørgensen, P.: Murine helix-loop-helix transcriptional activator proteins binding to the E-box motif of the Akv murine leukemia virus enhancer identified by cDNA cloning. Mol. Cell. Biol. 12, 3449-3459 (1992)

Pedersen, L., Behnisch, W., Schmidt, J., Luz, A., Pedersen, F.S., Erfle, V. and Strauss, P.G.: Molecular cloning of osteoma-inducing replication-competent murine leukemia viruses from the RFB osteoma virus stock. J. Virol. 66, 6186-6193 (1992).

Nørby, P.L., Pallisgaard, N., Pedersen, F.S., and Jørgensen, P.: Determination of recognition sequences for DNA-binding proteins by a polymerase chain reaction assisted binding site selection method (BSS) using nitrocellulose immobilized DNA binding protein. Nucl. Acids Res. 20, 6317-6321 (1992).

Pallisgaard, N., Nielsen, A.L., Pedersen, F.S., Birkelund. S. and Jørgensen, P.: A common poly-linker in a set of vectors for expression of eukaryotic genes in mammalian and insect cells and in Escherichia coli. Gene, in press (1993).

Lund, A.H., Duch, M., Lovmand, J., Jørgensen, P. and Pedersen, F.S.: Mutated primer binding sites interacting with different tRNAs allow efficient murine leukemia virus replication. J. Virol. 67, in press (1993).

Sørensen, A.B., Duch, M., Jørgensen, P. and Pedersen, F.S.: Amplification and sequence analysis of DNA flanking integrated proviruses by a simple two-step polymerase chain reaction method. J. Virol. 67, in press (1993).

Duch, M., Paludan, K., Lovmand, J., Pedersen, L., Jørgensen, P. and Pedersen, F.S.: A correlation between dexamethasone inducibility and basal expression levels of retroviral vector proviruses. Nucl. Acids Res. 21, in press (1993).

Head of project 6: Dr. Höfler

II. Objectives for the reporting period

The tumor suppressor p53 has been shown to be mutated in many different forms of human and animal cancers. In radiation-induced osteosarcomas evidence has also been accumulating showing frequent mutation at the p53 locus (see reports by IMV and GSF-P, this contract). Our long-term objective is to describe the radiation-induced molecular events leading to carcinogenesis. To achieve this we have committed much effort into establishing the methodological basis for both prospective and retrospective analysis of genetic alterations affecting key regulatory genes (tumor suppressors and oncogenes). In the next phase of the study we will begin to apply the analytical methods to materials provided by our collaborators. These include radiation-induced osteosarcomas (GSF-P and IMV) and colonies isolated from irradiated bone marrow.

III. Progress achieved including publications

Studies carried out in collaboration with GSF-P during the preceding CEC contract (B17-0002) have established the feasibility of molecular biological analysis of genomic material obtained from small tumor samples, including formalin-fixed and paraffin-embedded materials. We anticipate that the application of these molecular diagnostic procedures in our own contract, and in other studies in related programmes, will necessitate a high-throughput and inexpensive system. In addition we have attempted to design methodology that does not require radionuclides. In our preliminary studies to validate existing methodologies we have used reverse transcription and polymerase chain reaction to amplify p53 cDNA from tumor and normal tissue. Direct sequencing of the PCR product was used to ascertain the presence of mutations in the cDNA. These techniques have already been applied by ourselves and partners in the analysis of p53 mutations in cultured radiation-induced osteosarcoma cell lines (see report from IMV and GSF-P). However, their use in assaying archival material has not been tested.

In two studies we have established the methodology using human tumor materials (breast and lung carcinomas) where mutations were to be expected. We were able to demonstrate the effectiveness of the p53 analytical system using these test materials. In a sample of 28 small cell lung cancers mutations were found in 17

individuals, a frequency in accord with other published studies of p53 mutational frequency in cancers. The material used for this study was paraffin-embedded formalin-fixed, showing that the methodology is capable of detecting alterations in archival materials such as those to be made available in the EULEP archive.

This approach to p53 analysis proved superior to the previous screening strategy employing monoclonal antibodies against p53 to detect abnormal overexpression of p53 in tumor cells (see table 1). In a total of 23 cases of mammary tumors examined immunostaining analysis twice produced false positive results (eg case 1) and once a false negative result (case 3).

	p53 mutation by sequencing	p53 expression by immunohistochemistry
Case 1	wild type	overexpressed
Case 2	mutation	overexpressed
Case 3	mutation	not expressed

The single-strand conformational polymorphism assay (SSCP) (Orita et al 1989 Proc Natl Acad Sci 86: 2766-2770) has been shown to be a reliable method for screening large samples of nucleic acid for mutations. We have established the methodology using non-radioactive technology, and have shown that the SSCP is capable of detecting point mutational events in murine tissue samples. Testing of a known polymorphic site in exon 7 of the murine p53 gene (Bienz etal.1984 EMBO J. 3: 2179-2183) revealed that the assay was able to discriminate in 100% of cases the polymorphism in Balb/c and NMRI strain mice.

Publications:

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Lohmann D., Ruhri, Ch., Schmidt M., Graeff H., Höfler H. Accumulation of p53 protein as an indicator for p53 gene mutation in breats cancer Diagnostic Molecular Pathology 2 36-41 1993

Head of project 7: Dr. Schoeters

II. Objectives for the reporting period

The goals of our contribution are the identification of cells at risk for bone tumor development and the quantification of radiation or induced cellular and molecular alterations in bone marrow target cells at early stages in radiation induced bone tumour formation.
In vivo alpha-irradiation studies with osteosarcomogenic doses of ^{241}Am have demonstrated that bone marrow stromal cells are target cells which show long lasting changes in their osteogenic differentiation capacity (bone formation *in vitro*)
To identify stromal bone marrow cells among bone marrow further phenotyping has been done. Whether the changes are due to direct effects of alpha-particle irradiation, and whether they can be reproduced by *in vitro* alpha-irradiation has been studied. The ultimate goal is to develop an *in vitro* transformation assay by alpha-irradiation.

III. Progress achieved including publications

1. Characterization of bone precursor cells among bone marrow.

An enrichment procedure for osteoprogenitor cells was developed based on *in vivo* treatment with 5-Fluorouracil (5-FU) with the intention to determine the frequency, biophysical characteristics e.g. density, adhesion and lectin binding and the morphology of osteoprogenitor cells of the bone marrow.
In the presence of beta-glycerophosphate and vitamin C, cultures of normal mouse bone marrow cells form three dimensional structures which stain positive with the Von Kassa technique and express alkaline phosphatase (ALP), collagen Type I and ostocalcin. Most likely, mature osteoblastic cells do not contribute to the nodule formation since no osteocalcin expressing cells were detected in the flushed marrow by *in situ* hybridisation. Limiting dilution analysis demonstrated that in normal bone marrow, 1 out of 2.2×10^5 cells has the potency to form a bone nodule and to express ALP, collagen and osteocalcin in a temporal fashion. Upon *in vivo* treatment with 5-Fluorouracil (5-FU), this frequency increased 12 fold, e.g. 1 in 1.75×10^4 cells showed osteogenic activity. In comparison, fibroblast colony forming cells occurred at a frequency of 1 out of 2.5×10^4 or 1 out of 5×10^3 plated cells in normal or 5-FU treated marrow, respectively. Using density centrifugation, the majority of the osteoprogenitor cells in 5-FU marrow were found in the low density (1.066-1.067 g/ml) fractions. In addition these cells bound to nylon wool but not to plastic and aggregated in the presence of wheat germ (WGA) and soybean (SBA) agglutinin. Scanning and transmission electron microscopy showed that the bone nodules in 5-FU marrow cultures were composed of cells with a homogeneous fibroblastoid morphology which were embedded in a mineralised collagen matrix.

2. Effects of *in vivo* exposure of bone marrow cells to alpha-particle irradiation from ^{210}Po

Murine bone marrow contains osteogenic precursor cells which undergo differentiation during *in vitro* cultivation. Due to alpha-irradiation *in vivo* these cells are potential target cells for bone tumor formation.
Direct effects of alpha-particle irradiation from the radon daughter ^{210}Po have been studied using bone marrow cultures during their osteogenic differentiation. Polonium deposits in soft tissue and was shown to be associated with marrow cells and with the extracellular marrow tissue formed *in vitro*. These primary cultures exhibit high sensitivity to chronic alpha-particle irradiation. Cell death was obsersved at ^{210}Po concentrations in the tissue culture medium 10 times below those which cause general cytotoxic damage to undifferentiated cell line or to an osteogenic cell line. General cytotoxicity refers to non-specific damage which would occur in any kind of cell exposed to alpha-particle irradiaton provided the cells are actively dividing. House keeping cell functions are the targets. Comparison between effects on cell lines and on primary cell cultures indicate to what extent the specific properties of the putative target will alter quantitatively or qualitatively toxic effects. It can thus be concluded that primary osteogenic bone marrow cell cultures have properties which make them specific targets for direct effects of alpha-particle irradiation.
At lower concentration (between 1 and 5 Bq ^{210}Po per ml TCM) proliferation and expression of osteogenic differentiation markers (alkaline phosphatase activity, collagen synthesis) were enhanced in the bone marrow cultures.
It took some time before the changes became manifest. Growth of stromal stem cells (CFU-f) was not quantitatively altered but the proliferation of their descendants and the expression of osteogenic differentiation markers appeared sensitive. If ^{210}Po was added only the third and fourth week of cell culture, radiation induced changes were even more pronounced. At this time in culture, cell proliferation, alkaline phosphatase activity and collagen synthesis are rapidly increasing, perhaps making this stage especially radiation sensitive.
This study clearly shows that osteogenic marrow cells are direct targets for low density alpha-particle radiation. The radiation effects are specific for these cells and not due to general cytotoxicity.
Similar changes to osteogenic marrow cells have been noticed if mice were injected *in vivo* with ^{241}Am (Schoeters et al. 1992). Persistent damage to the osteogenic cells was than reported using a similar *in vitro* system but exposing cells *in vivo*. In a much shorter time the presented *in vitro* model system allows to observe the direct consequences of alpha-irradation to target cells. The underlying mechanism of these changes and their relation to transformation will be the subject for further study.

Publications

G. Schoeters, H. Leppens, U. Van Gorp and R. Van Den Heuvel.
Haemopoietic long-term bone marrow cultures from adult mice show osteogenic capacity *in vitro* on 3-dimensional collagen sponges.
Cell Prolf. (1992), 25, 587-603.

G. Schoeters, F. Vander Plaetse and R. Van Den Heuvel.
High radiosensitivity of the mineralization capacity of adult murine bone marrow *in vitro* to continuous α-irradiation compared to acute X-irradiation.
Int. J. Radiat. Biol. (1992), 61(5), 675-683.

G. Schoeters, F. Vander Plaetse, H. Leppens and R. Van Den Heuvel.
Response of murine stromal bone marrow cultures to α-particle irradiation *in vivo* and *in vitro* at osteosarcomogenic α-radiation doses.
Toxic in vitro (1993) (in press)

Progress Report

Contract: FI3P-CT920008 Sector: B21

Title: Research on the management of accidentally radiation exposed persons.

1) Fliedner Univ. Ulm
2) Wagemaker Univ. Rotterdam - Erasmus
3) Covelli ENEA
4) Jammet CIR

I. Summary of Project Global Objectives and Achievements

The 4 collaborating groups in the contract FI3P-CT920008 have agreed to execute a collaborative scientific programme on the research on the management of accidently radiation exposed persons. The objectives were to perform research relevant to 4 major areas: 1. **Pathophysiology** of radiation induced alterations of the immuno-hematopoietic system, of the respiratory and gastrointestinal systems and of the central nervous system. 2. **Diagnosis** of the radiation syndromes: preclinical and clinical approaches. 3. **Prevention** of the radiation syndromes and therapy of the radiation syndromes. 4. **Therapy** of the radiation syndromes.

In the first field of **pathophysiology of radiation induced alterations of different organ systems** it was the objective of the French group under directorship of Professor Jammet to study the radiation induced depression of different cell lines of blood cell formation with particular emphasis on interaction of stem-cells and stroma cells. This group also suggested to study the possibilities and limitations to tolerate single, repeated radiation exposures in relation to dose and dose rate. Further this group indicated the objective to study the development of radiation induced changes of the Central Nervous System under the particular consideration of neurological and psychic consequences.

The working group in the Netherlands directed by Professor Wagemaker suggested to study further the radiation sensitivity of hemopoietic stem cells and to examine carefully the further possibilities of influencing the LD50 of bone marrow damage by new approaches of intensive care.

The group in Italy directed by Professor Covelli had as its major objective to study the influence of ionizing radiatin on functions of lymphocyte populations.

Finally the group in Germany directed by Professor Fliedner suggested to study further the replication and differentiation of hemopoietic stem cells after total and partial body irradiation, the kinetics of hemopoietic reconstitution after total and partial body irradiation using especially new biomathematical models to simulate pathophysiological courses of events after total body irradiation with the objective to assess the remaining and initiating stem cell pool.

With respect to the further improvement of **diagnostic possibilities of the radiation syndromes** the French group suggested to study indicators of total and partical body irradiation considering particularly the efficiency of growth factors and of models relating biological indicators and physical dosimetry. The Dutch group agreed to study the stimulation of hemopoiesis by hemopoietic growth factors as a test system for the capacity of autochthonous regeneration. The Italian group wanted to further develop biological indicators to evaluate the radiation induced damage of the immune system and to predict the pathological

consequences. The group in Germany suggested to study the relevance of blood cell changes in quantity and quality as prognostic indicators for or against stem cell transplantation. These studies will also be of relevance to answer the question whether regeneration of radiation induced hemopoietic damage can be enhanced by the use of recombinant stimulation factors.

As far as the **prevention of radiation syndromes** is concerned the French group suggested to use enzymes to prevent the development of fibrotic changes and of vascular late effects. The Dutch group suggested to study hemopoietic growth factors as radiation protectors while the Italian group suggested to study recombinant cytokines as radiation protectors. The group in Germany laid major emphasis on the research to establish stem cell banks for persons at risk. Here the major emphasis is now on the purification of stem cells from the peripheral blood for allogeneic transplantation.

As far as the **therapy of the radiation syndromes** is concerned the French group suggested to study further methods for the inhibition of graft versus host disease including selective elimination of lymphocytes to improve the use of stem cells for the reconstitution of hemopoiesis by comparing stem cells from the bone marrow with those of the blood especially of the cord blood. The French group also suggested to use hemopoietic growth factors to improve the reconstitution of hemopoiesis. The Dutch group is particularly devoted to research on the acceleration of autochthonous regeneration by hemopoietic growth factors on the improvement of the possibilities to replace conventional intensive supportive care by specific combination of hemopoietic growth factors and by studying the transient engraftment of purified stem cells in cases where the spontaneous recovery of hemopoiesis is in doubt. The Italian group performs research on the modulation of the immune response by recombinant cytokines and also with the improvement of the rapidity in the HLA typing preparing for bone marrow transplantation. The group in Germany is concerned with particular studies on the treatment of dogs with recombinant factors to enhance the recovery of the megakyrocyte platelet system. This group also suggested to work closely together with a group in the United States to study further possibilities and limitation to mobilize stem cells into the peripheral blood, to use cord blood cells for allogeneic transplantation and to influence hemopoietic recovery by growth factors.

As far as the **achievements** of the studies are concerned, the individual laboratory reports give detailed information. It can be seen from the group reports that all participating groups contributed to a large extent to the objectives stated. However, it should also be pointed out that due to the restriction of financial resources for scientists and for carrying out the necessary scientific studies each group had to concentrate on some aspects of the stated objectives and was not able to perform all the studies envisaged. However, in the next reporting period the studies will be continued and expanded in those areas which originally were felt to be of great importance for the success of this collaboration.

It should also be stated that the group met in 1993 (May) in the University of Ulm to exchange in detail the results of progress achieved and to stimulate a further collaboration. In 1994 the series of collaborative scientific workshops of the group will be continued in Paris in order to make sure that the scientists of the participating groups will continue to perform joint studies or coordinated activities.

Head of project 1: Prof. Fliedner

II. Objectives for the reporting period

The Ulm team of the European working group supported by the EC-Contract No. FI3P-CT92-0008 concentrated its work during the period of May 1, 1992 - August 30, 1993 on 4 major areas: **1**. Pathophysiology of radiation induced alterations of the immune-hemopoietic systems considering in particular replication and differentiation mechanisms of hemopoietic stem cells, **2**. Diagnosis of the radiation syndromes using biomathematical models for cell system alteration analyses, **3**. Prevention of the radiation syndromes by examining potentialities of blood stem cell banks not only for autologous but also for allogeneic bone marrow restaurations and **4**. Treatment of the radiation syndromes by studying clinical approaches for hemopoietic reconstitution by blood derived stem cells.

Objectives for the next reporting period:

For the next reporting period it will be the objectives to go further in the study of the replication and differentiation of hemopoietic stem cells in human beings as well as in the canine model to better understand the possibilities of influencing the recovery of the hemopoietic system by recombinant factors. In addition we are proposing to continue the efforts to simulate by biomathematical modelling the kinetics not only of granulopoiesis but also of megakaryocytopoiesis, lymphopoiesis and erythropoiesis under the influence of acute and chronic radiation. Furthermore we are proposing to develop further an expert system for the advice of doctors treating radiation injured patients using available data from radiation accident management experience. In addition we are proposing to study DNA damage by means of single cell gel electrophoresis in order to improve methods for detecting reparable and irreparable damage to the hemopoietic stem cell.
The Ulm group will in addition collaborate with groups in the United States to develop a purified blood stem cell bank for allogeneic transplantation purposes. Finally the Ulm group will continue to study recombinant hemopoietic growth factors in order to enhance hemopoietic recovery of the megakaryocytic system. In addition further studies will be done to use blood derived stem cells for the treatment of hemopoietic failure not only under autologous but in particular allogeneic conditions.

III. Progress achieved including publications

The results attained by the Ulm group in the framework of the stated objectives can be summarized as follows:

1. Pathophysiology of radiation induced alterations of the immune-hemopoietic system

In order to characterize further replication and differentiation of stem cells, it was the goal of the studies performed to study 2 different progenitor populations ($CD34^+DR^+$ and $CD34^+DR^-$) isolated from cord blood (CB). The FACS analysis shows that about 1% of the CB cells ($\leq$ 1.077 g/ml) express the CD34 antigen. Two parameter FACS analyses were performed for the examination of co-expression of HLA-DR antigens on $CD34^+$ cells. Based on these studies we were able to proof the existence of a small progenitor cell population with the phenotype $CD34^+$ $HLA\text{-}DR^-$. This is in contrast to other reports who postulated that all CD34 positive

progenitor cells from cord blood should carry the DR antigens. Therefore, the studies were expanded as follows:

In short-term clonogenic assays in the presence of IL-3, GM-CSF, hSCF and Epo or combinations thereof the $CD34^+$ $HLA\text{-}DR^+$ population were shown to have a higher clonogenic potential than the $CD34^+$ $HLA\text{-}DR^-$ population. However, using a more stringent stem cell assay, i. e., CFU-blast assay, the $CD34^+$ $HLA\text{-}DR^-$ cells but not the $CD34^+$ $HLA\text{-}DR^+$ cells gave rise to CFU-blasts that, upon replating, generated secondary colonies of different cellular composition. Notably single cell cDNA-PCR analysis of immunomagnetically isolated or sorted (purity>99%) $CD34^+$ $HLA\text{-}DR^-$ cells demonstrated that these cells express HLA-DR at the mRNA level.
Our data indicate that $CD34^+$ $HLA\text{-}DR^-$ cells represent a more primitive hemopoietic cell population than their $CD34^+$ $HLA\text{-}DR^+$ counterparts. Furthermore the cDNA-PCR analysis indicates that $CD34^+$ $HLA\text{-}DR^-$ cells express HLA-DR at a low level which is undetectable by FACS/immunomagnetic beads or that HLA-DR transcripts are not translated into their protein products.

As far as the radiation effect studies on stem cells is concerned, the following results were obtained:

Human multipotent hemopoietic cells could be isolated and enriched by means of monoclonal antibodies against surface differentiation antigens. Human cells expressing CD34 antigen virtually comprise hemopoietic progenitors forming in vitro multilineage colony forming units (CFU-mix), erythroid burst (BFU-E) and granulocyte-macropohage colony forming cells (GM-CFC). In the present study $CD34^+$ cells were isolated from human cord blood and irradiated with increasing doses of 280 kV x-rays (72 cGy/min). The surviving fractions of irradiated progenitors including CFU-mix, BFU-E and GM-CFC were determined in cultures containing erythropoietin (Epo) and placenta conditioned medium (PCM), both needed for optimal colony growth. The radiosensitivity of $CD34^+$ cells was also studied in cultures containing additionally to Epo and PCM recombinant human Stem Cell Factor (hSCF) or Basic Fibroblast Growth Factor (b-FGF). The obtained D_0 values show that hSCF increased markedly the clonogenicity of all progenitors compared to cultures stimulated only with PCM. In contrast, b-FGF showed only a weak radioprotective effect.
Canine hemopoietic progenitors are the most radiosensitive mammalian hemopoietic cells. However, at present it is not possible to separate canine multipotential progenitors as it is the case for human $CD34^+$ cells. The influence of hSCF and b-FGF on the radiosensitivity of hemopoietic progenitors in canine bone marrow was studied using gradient enriched cell fractions. Canine cells were cultured immediately after irradiation with Epo and post-irradiation dog serum (PIDS) which contains factors needed for optimal growth of CFU-mix and BFU-E. The addition of hSCF to the cultures of irradiated cells increased markedly the D_0 value of the progenitors. The radioprotective effect of b-FGF was much lower, similarly as with human cells.
The CFU-F (colony forming units -fibroblast) are the progenitor cells of the hemopoietic stroma. Together with several other types of cells they form the hemopoietic microenvironment. Canine CFU-F are relatively radioresistent (D_0=2.61 Gy). Preliminary results showed improved colony formation of irradiated CFU-F in the presence of b-FGF but not hSCF.

2. Diagnosis of the radiation syndromes

With the help of a "sequential diagnosis" it is possible to assess the extent of damage after ionizing whole body irradiation relevant for the execution of therapeutic measures. The key question to be answered is whether one is dealing with a "reversible" or "irreversible" damage to the organism as expressed in the hemopoietic system. Using a biomathematical simulation model of granulocytopoiesis consisting of bilinear subsystems which are connected by non-linear static transfer elements, it is now possible to use the early (first 3-5 days) granulocyte changes in the blood after total body exposure to predict the outcome. This new model allowed us to analyse in detail 6 patients who were involved in severe radiation accidents. The granulocyte simulation model was able to show that the granulocyte concentration changes in the peripheral blood during the first 5 days after the accident are compatible with the assumption of virtually no surviving intact stem cells and a few surviving injured stem cells. In contrast, the analysis of 4 patients that were subjected to irradiation doses in excess of the "LD50" for human beings, shows that it is possible to derive from the early granulocyte pattern change the confirmation that some stem cells survived making an spontaneous recovery possible. In the model, the fraction of surviving stem cells must be in the order of more than 6 per 10,000 to allow a spontaneous hemopoietic recovery. Thus, it is now possible to predict from early blood granulocyte changes whether a "reversible" or "irreversible" hemopoietic damage has occured or not.

This model has also been used to analyse the effects of chronic low level exposure to dog (0.4 cGy/day to 17.0 cGy/day: in collaboration with the Argonne National Laboratory, USA) on the hemopoietic system. A "turbulance region" was defined with respect to the relative kill rate of hemopoietic stem cells. If one remains below a certain dose rate, the system is able to tolerate low level exposure without failure of the hemopoietic system in spite of the damage inflicted on the stem cell population. If it is within or above this "turbulance region", then the likelyhood of a hemopoietic failure increases dramatically.

3. Prevention of the radiation syndromes

In close cooperation with Dr. M. Körbling from M. D. Andersen Hospital, Texas, the studies were continued with respect to the definition of the appropriate conditions for mobilizing, collecting, purifying and using stem cells from the peripheral blood for the restauration of hematopoiesis after bone marrow failure. The objective of our studies was to establish a large-scale technique for purification of $CD34^+$ DR^- progenitor cells from a large volume marrow harvest. In five different experiments, as a first step $CD34^+$ cells were purified to between 76% and 91% by avidin-biotin immunoadsorption (CEPRATE SC). This was followed by fluorescence activated cell sorting (Becton-Dickinson) to separate DR^+ and DR^- cells which resulted in the generation of between 1.75 and 11.3 x 10^5 $CD34^+$ DR^- cells. The purity of DR^- cells increased from between 0.5% and 4.3% in the immunoadsorbed fraction up to 99% in the DR^- sorted fraction. We were able to physically separate one $CD34^+$ DR^- cell from up to 8,000 nucleated cells in the pre-purified cell suspension. One million highly purified $CD34^+$ DR^- progenitor cells is potentially an adequate cell dose for autologous transplantation equivalent to what is contained in an unselected and functioning marrow autograft.

These studies will now be expanded to attempt the collection, isolation and use of pluripotent hemopoietic stem cells in the allogeneic situation.

4. Therapy of the radiation syndromes

The experimental studies during the last year concentrated on the question, whether it is possible to modulate the effects of whole body irradiation on the megakaryocyte-platelet systems by the administration of cytokines.

IL-6 has been shown to stimulate megakaryocyte formation and function in in vitro models and also to enhance platelet production in experimental animals with normal hemopoietic function in a dose-dependent fashion. This suggests its possible therapeutic use to stimulate the recovery of megakaryocytopoiesis from radiation induced damage. Therefore we have tested the hematological effects of recombinant human IL-6 (rhIL-6, SANDOZ PHARMA AG, Basel/Switzerland) in dogs after total body irradiation (TBI) with a dose of 2.4 Gy. RhIL-6 was administered over a period of 14 days at a daily dose of 18 ug/kg by single subcutaneous injections. Treatment was started 1 day after TBI was performed. The values obtained for different hematological parameters in the irradiated rhIL-6 treated dogs were compared with the corresponding data obtained from 2.4 Gy total body irradiated dogs which received treatment with the carrier (controls) instead of rhIL-6. When compared to the irradiated control animals no influence of the rhIL-6 treatment on the temporal pattern of recovery of the lymphocytes and the monocytes could be observed. On the other hand, in the rhIL-6 treated animals the neutrophilic granulocytes tended to show a certain delay before their concentration reattained the normal pre-irradiation range. The temporal pattern of changes in the thrombocyte counts in the period from day 1 to day 16 after TBI was similar in the dogs of both groups, showing a sharp decrease from day 6 to day 12 and a stabilization thereafter at minimum levels of approximately $20x10^3$/ul blood. However, in the rhIL-6 treated animals the thrombocyte counts commenced to increase at day 17 after beginning the treatment (with certain exceptions), i. e. by 7 days earlier than in the nontreated controls and also reattained their normal levels by 7 days earlier than in the latter. On the other hand, rhIL-6 treatment was associated with a certain decrease in the blood erythrocyte counts by approximately 2% per day during the whole treatment period in addition to the radiation-induced reduction. The results of these studies show that under certain circumstances treatment with rhIL-6 may be useful to specifically stimulate the recovery of megakaryocytopoiesis from radiation-induced damage. However, the possible adverse effect of rhIL-6 on erythropoiesis has to be taken into account in cases with severe bone marrow damage.

5. Publications

1. Fliedner, T. M., Nothdurft, W. Simulationsmodelle der Hämatopoese zur Abschätzung der Strahlenbeanspruchung bei fraktionierter oder chronischer Strahlenbelastung. In: Kreutz, R. und Piekarski, C. : Verhandlungen der Deutschen Gesellschaft für Arbeitsmedizin e. V., Gentner Stuttgart, 1992.

2. Fliedner, T. M., Heinze, B., Plappert, U. Biologische Indikatoren zur Evaluation der Beanspruchung des Organismus durch ionisierende Strahlen. In: Kreutz, R. und Piekarski, C. : Verhandlung der Deutschen Gesellschaft für Arbeitsmedizin e. V., Gentner Stuttgart, 1992.

3. Fliedner, T. M., Nothdurft, W., Tibken, B., Hofer, E. P., Weiss, M., Kindler, H. Hemopoietic Cell Renewal in Radiation Fields. Proceedings of the Congress (XXIX COSPAR Plenary Meeting and Associated Activities World Space Congress 28.08.92-09.09.92 Washington), in press.

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23. Thoma, S., Lamping, C., Ziegler, B. Phenotype Analysis of Hematopoietic $CD34^+$ Cell Populations derived from Human Umbilical cord Blood using Flow Cytometry and cDNA-PCR. Submitted to Blood 1993.

RADIATION INDUCED DAMAGE AND RECOVERY OF THE HEMATOPOIETIC SYSTEM

(Rotterdam, The Netherlands)

Aims for the past period (May 1, 1992 - August 31, 1993):

The program of the Rotterdam group is directed at radiation induced damage and recovery of the hematopoietic system to improve diagnosis and treatment of accidentally irradiated individuals and to facilitate the initial treatment choices that need to be made, i.e., conventional treatment by supportive care, electively supplemented with growth factor treatment, and or bone marrow transplantation using highly purified stem cells. The research projects make use of rhesus monkeys as the primary experimental model. In the past period, emphasis has been laid on the further development of the use of growth factors, in particular IL-3 and IL-6, which have been fully characterized in their biological effects and side effects as well as efficacy. Similar studies with SCF are in progress. Focus has also been placed on the use of highly purified stem cells defined as CD34-positive cells. In addition, the monitoring of growth factor receptor regulation by flow cytometric detection using biotinylated growth factors has been further developed for erythropoietin, IL-3, SCF, GM-CSF and IL-6. It is now routinely possible to monitor these growth factor receptors during bone marrow regeneration after total body irradiation.

Aims for the next period (September 1, 1993 - May 31, 1994)

In the forthcoming period the project will focus on delineating synergistic and/or additive effects of the three growth factors IL-3, IL-6 and SCF in irradiated rhesus monkeys as a start to design an optimal combination therapy to accelerate hemopoietic reconstitution. These growth factors were selected for their known stimulation of the most immature cells presently detectable. In addition, we expect to further subfractionate the CD34 positive cells to come closer to the identification of the stem cell type responsible for long term reconstitution after bone marrow transplantation. This is done using beads methods for large-scale separation as well as dual laser, eight parameter cell-sorting. Identification of this cell type and its growth factor responsiveness and phenotype is thought to be of pivotal importance for the development of a rational approach towards growth factor supported bone marrow regeneration after irradiation.

Progress report past period (May 1, 1992 - August 31, 1993)

A major problem in the management and treatment of heavily irradiated victims of a radiation accident is the identification of patients that will recover spontaneously following conventional treatment and those that will need a bone marrow transplantation for recovery. Studies on the radiation sensitivity of primates have led to a reappraisal of the radiation sensitivity of stem cells. A D_0 value of 0.9 Gy for X-rays is now assumed rather than 0.6 Gy as was earlier calculated. Accordingly, a residual number of hemopoietic stem cells capable of autogenous regeneration can now be assumed up to a dose of 10 Gy TBI, at which gastrointestinal damage will become problematic. This hypothesis has been prospectively tested in a small number of rhesus monkeys using extensive supportive care (gastrointestinal decontamination, antibiotic treatment, fluid and electrolyte administration and deliberate transfusions of blood products). The results indeed demonstrated that even at high doses of TBI, hemopoiesis will ultimately (6 to 8 weeks after TBI) regenerate and that the $LD_{50/30d}$ of about 5 Gy hitherto reported will be shifted to about 9-10 Gy. By applying this technology, the rhesus monkey model has become suitable to study radiation induced damage and recovery of the hemopoietic system up to TBI doses of 9 Gy. This model is currently used to further develop new treatment strategies.
Firstly, earlier work in rhesus monkeys and other animal models has shown, that hemopoietic growth factors up to certain doses of TBI will prevent or shorten the duration of pancytopenia. For instance, monotherapy with GM-CSF (granulocyte/macrophage colony-stimulating factor)

prevents granulopenia at a dose of 5 Gy TBI, shortens its duration at 6 Gy TBI, but is ineffective at doses larger than 7 Gy TBI. In the reporting period, similar studies have been completed for IL-3 and IL-6, directed at finding optimal doses, efficacy following total body irradiation as well as a full account of the encountered side effects. The studies on IL-3 can be briefly summarized as follows.The full stimulatory spectrum of IL-3 was tested in a preclinical rhesus monkey model. Recombinant homologous IL-3 was used, as human IL-3 was shown to be insufficiently effective in stimulating the rhesus monkey hemopoietic system. Administration to normal rhesus monkeys resulted in a dose-dependent stimulation of the production of all major bone marrow derived peripheral blood cells, after a lag phase of one week in which an increase of progenitor cells in the bone marrow was observed. Peripheral blood T and B lymphocyte numbers remained unaffected by IL-3 treatment. Characteristic for IL-3 treatment was the production of high numbers of atypical basophilic cells, which comprised the majority of nucleated cells at the end of IL-3 treatment, and a concomitant high level of histamine. At low dose levels, thrombocytosis was observed, whereas high dose IL-3 treatment resulted in severe thrombopenia. Normoblastosis preceded reticulocytosis, but did not result in a rise in erythrocytes or hemoglobin levels; at high doses, anemia was seen. Pharmacokinetics of (recombinant nonglycosylated) IL-3 predicted that the intravenous route of administration should be more effective than the subcutaneous route of administration, which was confirmed by comparison of both treatment modalities. A neutralizing antibody response was elicited by high doses of IL-3. Prolonged iv administration of low dose IL-3 did not result in antibody formation.

To evaluate the mechanisms involved in the species specificity of human IL-3, binding studies using radiolabeled IL-3 were performed. The rhesus monkey IL-3 receptor complex bound homologous IL-3 20- to 50-fold higher more effectively than human IL-3, whereas human IL-3 receptors bound both species of IL-3 with similar affinity, demonstrating the unidirectional species specificity of human IL-3. The species specificity of human IL-3 was confirmed by in vivo comparison of both species of IL-3; homologous IL-3 was about 5-fold more effective than its human counterpart in stimulating hemopoiesis.

Since little information was available on IL-3 receptor expression of normal bone marrow cells, the binding characteristics of the IL-3 receptor were evaluated by conventional binding studies using radiolabeled IL-3. The distribution of IL-3 receptors on different cell types was investigated by more novel flow cytometric methods, using biotin-labeled IL-3. An average of 50 high- and 1100 low-affinity IL-3 receptors were detected on normal rhesus monkey bone marrow cells. Competition of high-affinity IL-3 binding by GM-CSF could not be demonstrated, probably due to differential expression of growth factor specific a-chains. IL-3 receptors could only be demonstrated on some 3% or less oft he isolated CD34-positive bone marrow cells, which did not express c-kit. When rhesus monkeys were treated with IL-3, the number of high- as well as low- affinity IL-3 receptors did increase significantly, which could be attributed to the increase of atypical, histamine containing, basophilic cells. The percentage and absolute number of CD34-positive, c-KIT-negative cells, expressing IL-3 receptors did not increase during IL-3 treatment.

Besides the hemopoietic effects, a variety of dose dependent side effects were observed during IL-3 treatment, which included urticaria, edema, enlargement of spleen and lymph nodes and at high dose levels, anemia and thrombopenia. Irrespective of the dose, acute arthritis was observed in several animals. This type of arthritis was not prevented by the MHC allele A26, which counteracts the development of collagen-induced arthritis in juvenile monkeys. The MHC alleles B9 and Dr5 were significantly more frequently found in the animals which developed IL-3-induced arthritis. Side effects could not be prevented by the addition of histamine antagonists to the treatment protocol, most probably due to perpetual production and activation of IL-3R-expressing, atypical basophils, resulting in a continuous release and subsequent high levels of histamine.

The effect of IL-3 on bone marrow after cytoreductive treatment was tested in animals subjected to 5 Gy TBI. IL-3 treatment before 5 Gy TBI resulted in a later onset of a reduction in reticulocytes, whereas no effect was seen on the onset of leukopenia or thrombocytopenia. Pretreatment with IL-3 did not result in a more rapid recovery from pancytopenia; a delay in accelerated expansion of reticulocytes and thrombocytes was observed, whereas the leukocyte regeneration was comparable to that of control irradiated animals. IL-3 treatment after TBI resulted in shortening of the pancytopenic phase, more prominent for the red cell and

thrombocyte lineages than for granulocytes. Remarkably, IL-3 stimulated the recovery of neutrophilic granulocytes rather than that of basophils and eosinophils. A decrease in platelet and red cell transfusion requirements was observed. Serial measurements of colony-formation by bone marrow CFU-GM, BFU-E and CFU-E demonstrated accelerated bone marrow recovery, in agreement with the stimulated recovery of peripheral blood cells in animals treated with IL-3 after TBI. Side effects were only mild in myelosuppressed animals.
The effects of IL-3 in irradiated animals were more limited than predicted from the strong stimulatory effects of IL-3 in unirradiated animals. It was concluded that IL-3 may have a direct stimulatory effect on immature hemopoietic progenitor cells, albeit limited due to the low frequency of bone marrow cells expressing IL-3 receptors. The strong hemopoietic effects of IL-3 in normal rhesus monkeys may be mediated by increased production of other stimulators. Histamine, and possibly IL-1, IL-4,IL-6 and TNFa are candidates for these mediators. It remains to be investigated whether the rare CD34+/c-kit-/IL-3R+ bone marrow cell population has stem cell properties and whether the perpetual activation of IL-3 receptor expressing atypical basophilic cells have a bearing on the hemopoietic effects of IL-3.
A very similar study has been completed for IL-6 and demonstrated its efficacy in stimulating the thrombopoietic series much more prominently than could be achieved with IL-3 or GM-CSF. Side effects of IL-6 especially included fever and anemia due to sequestration of cells in liver and spleen, but were readily reversible upon discontinuation. For glycosylated IL-6 used, there was no difference between the iv or sc route of administration. IL-6 receptors were detected on a large proportion of CD34-positive cells. IL-6 stimulated the recovery of bone marrow cells as detected by GM-CFU and BFU-E assays, which may indicate that it might strongly synergize with more mature acting growth factors such as erythropoietin and G-CSF.

The development of CD34 positive cells for bone marrow transplantation has in the past period been pursued further subfractionation using DR-antigens and eliminating cells expressing antigens for T lymphocytes, as well as on the basis of expression of receptors for erythropoietin, IL-3, IL-6, GM-CSF and SCF. These studies are currently being done by culture assays for immature cells, on which basis specific subfractions of CD34 positive cells will be transplanted in rhesus monkeys, using retrovirally inserted marker genes for clonal analysis after transplantation, to establish their capacity for sustained, multilineage regeneration.

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Head of project 3: Dr. Covelli

II. Objectives for the reporting period

The project is focussed on radiation damage and recovery of the immune system, aimed at designing appropriate strategies for medical intervention in radiation accidents and radiotherapy.

The main objectives are:

1. Effects of IL-3 on the recovery of T and B lymphocytes in sublethally irradiated mice.

2. Improvement of serological and recombinant DNA techniques for rapid and precise HLA typing in humans.

III. Progress achieved including publications

Studies on mice. IL-3 is a colony-stimulating factor that regulates hemopoiesis. This cytokine, indeed, is involved in the differentiation of pluripotent stem cells to mature cells of several lineages, such as neutrophils, macrophages, erythrocytes, eosinophils, megakaryocytes and mast cells. IL-3 also promotes limited self-renewal of the multipotent stem cells which give rise to splenic colonies. We have investigated whether murine recombinant (mu r) IL-3 injected into mice exposed to sublethal irradiation can accelerate the recovery of thymocytes and splenic T and B cells. It was found that injection of mu r IL-3 into (C57Bl/10 x DBA/2)F1 mice, sublethally irradiated with 200 cGy and sacrificed 7 days later, induced in the thymus recovery of the cell number and mitotic responsiveness to Con A, as well as a decrease in double negative CD4-CD8- cells concomitant with an increase in single positive CD4+ and CD8+ cells. Also in the spleen, the cell count and mitotic responsiveness to Con A and LPS were increased to normal levels by mu r IL-3 treatment. If the assays were performed 14, 21 or 28 days after irradiation, mu r IL-3 treatment was able to restore thymus and spleen cell counts as well as T

and B cell mitotic responsiveness even when mice were exposed to 300, 400 or 500 cGy, respectively. These results altogether indicate that mu r IL-3 induces differentiation and growth of thymocytes and recovery of T and B cell functions in mice exposed to sublethal irradiation. Thus, IL-3 appears as a powerful molecule that can be successfully used in radiation accidents.

Studies on humans. Cell typing for bone marrow transplantation in irradiated persons requires rapid and precise techniques. In collaboration with Prof. G.B. Ferrara from the Cancer Institute in Genoa, human mAb and recombinant DNA techniques have been applied to the identification of HLA allelic specificities. A new series of mAb specific for HLA class I and II molecules has been produced while the use of digoxegenin has allowed a very rapid DNA typing with no need for radioactive probes. It has also been possible, by host and donor DNA typing, to detect bone marrow take as soon as 10 days after transplantation.

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Objectives for the next reporting period

As in 1992/93, research will be focused on problems related to damage and recovery of the immune system after radiation exposure. These aspects are of particular importance for the treatment of radiation accidents because they result in a decreased resistance to pathogens and in development of auto-immune diseases and cancer. The adopted experimental approach, using genetically homogeneous animals and standardized irradiation and treatment conditions, is providing reproducible data to evaluate the consequences of radiation accidents and to design appropriate strategies for effective medical intervention.

Mice will be total-body exposed to 200 - 500 cGy of X-rays and repeatedly injected with recombinant IL-11 starting immediately after irradiation. Mice will be sacrificed at weekly intervals after irradiation and their thymuses and spleens assayed for cellularity, thymocyte mitotic response to Con A, and splenocyte mitotic responses to Con A and LPS. Comparison of the effects of these treatments after different radiation doses should indicate the conditions for optimal intervention to accelerate recovery of the immune system and to prevent radiation death.

Head of project 4: Prof. Jammet

II. Objectives for the reporting period

Immunohematopoietic modifications after exposure to ionizing radiation was considered as a priority for improving the management of accidentally irradiated patients during the reporting period. Different approaches have been made, including :

1-Diagnosis of Radiation Syndrom : preclinical and clinical approaches :

-early clinical follow-up during and after a total body irradiation (TBI) resulting in a prospective evaluation of the prodromic phase.

-research on the time interval during which it remains possible to carry out HLA typing after high-dose TBI.

- biological Indicators for overexposure : evolution of the blood levels of CRF, ACTH, and cytokines after high and low dose TBI

2-Pathophysiology of radiation induced alterations:

- research on the molecular basis of radiation induced intermitotic cell death in human lymphocytes,
- characterisation of genes playing a key role in immunological tolerance,
- study of radiosensitivity of bone marrow precursor cells,
- research on the pool of rare cells following early hyperleucocytosis induced by radiation.

3-Therapy of the Radiation Syndromes:

- experimental study on the methods leading to an attenuation of GVHD (graft versus host disease) and graft rejection in mouse after MHC-haploincompatible bone marrow transplantation.

For the next incoming period of the contract we propose :

- to extend those analysis to other types of therapeutic TBI (low dose : 2 Gy) in view to point on the specific clinical and biological data that may be used for a rapid evaluation after an accident ,
- to study the modification of genes and mRNA involved in stem cell differenciation in irradiated patients.
- to study the genes involved in cellular sensitivity, notely those controling, apoptosis,
- to determine the differential distribution of blood cells progenitors (CD34, ckit, Thy1, HLA DR) in the blood of patients before and after TBI.
- to identify DNA damage inducible genes.

III Progress achieved including publications

1-In order to determine as early as possible the severity of a whole-body accidental overexposure, studies were performed on early follow-up (24 h) of patients having TBI (Total Body Irradiation) to point out the practical use of clinical and biological indicators. Such a study of the "prodromal phase" was performed on patients with haematological malignancies who received a high dose irradiation (10 Gy in 4

hours) 24 hours before high dose cyclophosphamide for bone marrow transplantation.These patients were able to be prospectively evaluated for clinical symptoms during and for a 20h period after irradiation.The most striking points are the high frequency of nausea and vomiting in patients not given a specific antiemetic (Ondansetron) and the demonstration of an almost constant temperature peak (40.8 °C) six hours after the start of a 10 Gy-4h TBI. An other point of interest is the frequency of parotidis complain with early xerostomia (61 % during irradiation) and parotidis pain in the first 24 h (74%).These points, together with the precise time-course of the other symptoms of the prodromal phase as headhache,ocular symptoms, blood pressure,respiratory and cutaneous signs, might be of some help for the preliminary triage of victims of an accidental irradiation.
A main question after an accidental TBI is to determine the indications of an allogeneic bone marrow transplantation and as the case may be, to identify HLA-compatible donors. In such a case, class I (A,B) and II (DR) HLA typing of the irradiated person must be precisely determined. The answer to two questions is needed : after a high dose total irradiation , is it possible to define HLA genotype and if so, how long after irradiation does a reliable HLA genotyping remain possible? this approach was performed on patients following TBI at high dose (10 Gy-γ ray) before bone marrow transplantation. Those patients had a complete HLA-typing before TBI and at different time after irradiation; the comparison of these results performed using conventional serologic technic, permitted us to define the reasonable delay of investigation(2). A reliable class I HLA typing appeared to be possible in almost all cases 6-8 hours after the start of irradiation but was only possible in few patients after 24 hours. Preliminary results with class II antigens might suggest a more marked fragility of this antigen class after irradiation. These results encourage the drawing of blood sample for HLA grouping as soon as possible after accidental TBI. The possibility of molecular biology techniques to overcome the severe limitations of the serological procedures is being carried on.

2-During the period of time of the contract a significant effort for developping molecular biology of the immunohematopoietic system following TBI has been initiated at Hopital St Louis and Institut Curie.
The molecular basis of radiation induced intermitotic cell death of human lymphocytes has been investigated. Several evidences have been established concerning a relationship between ubiquitin gene expression/nuclear protein ubiquitination and radiation mediated apoptosis in normal circulating human lymphocytes. (3)
Irradiated in vitro at 5 to 10 Gy with gamma rays, 70 to 85 % of human lymphocytes showed morphological changes characteristic of apoptosis at 24h of postirradiation culture. Following 5 Gy of γ-irradiation :
-the ubiquitin mRNA level is increased (4 to 5 fold) 15 to 90 minutes after initiation of apoptosis
-specifically in apoptotic cells nuclear proteins are highly ubiquitinated
-use of ubiquitin mRNA antisense sequences decreased level of ubiquitinated nuclear proteins and, consequently,
-the proportion of cells disclosing the apoptotic death pattern is significantly decreased (30 -35 % instead 70-85 %)
This results suggest that the ubiquitin gene be one of the "death" genes with induced activity in γ-ray mediated apoptosis and that the ubiquitination of nuclear proteins be

involved in chromatin disorganisation and fragmentation. Target inhibition/analysis of different ubiquitinated substrates should help to provide a better understanding of the molecular basis of the cell death induced by γ-rays.
Recent results have shown that stem cells from the cord could be a suitable source for bone marrow repopulation, under semi-allogenic conditions. These results may be extremely promissing after accidental TBI, allowing to overcome the difficulties raised, by finding rapidly a HLA compatible graft, and in some instances, by the rapid disappearance of HLA expression on irradiated lymphocytes membranes (2) which may make HLA typing difficult in the case of a severe accident. It has been suggested that non classical HLA-G gene plays a key role in immunological tolerance, therefore we have been looking at HLA-G expression in several tissue samples : Fetal trophoblasts, fetal liver cells, mononuclear cord blood cells, mononuclear adult blood cells. RT-PCR and Southern blots hybridization have shown that our primers were highly specific for HLA-G. HLA-G was found expressed in early trophoblasts and in adult mononuclear cells but only slightly in foetal cord blood and liver cells. DNA resulting from RT-PCR resulted in 2 bands (450 and 700 bp) in trophoblast and only one band in lymphocytes. This results from differential transcription of HLA-G gene. An interesting spin off of these study is the characterization of putative maternal cells in umbilical cord blood cells (manuscript in preparation)
It is generally accepted that multipotent precursor cells (CFU-GEMM, CFU mix) in human bone marrow have a D_o between 0.5 and 1.4 Gy. Stem cells are characterized by a CD 34+, Thy1+, Ckit+ phenotype. An approach of the radiosensitivity of these rare cells could be provided by KG1A cell line which showed a D_o of about 2Gy in our hands. We started therefore to separate CD34+ cells by immunoaffine magnetic beads. Our results have led to a pool of .9 - 3 x 10 6 cells whose 60% were CD34+.
Hyperleucocytosis is a well known phenomenon following TBI. Some progenitor cells may participate to this increase (CFU-GM). We have followed T cells : CD3, CD5, CD8, CD45 Ra and Ro; NK (CD 56, CD 16); B cells CD19 and CD34, Ckit, HLA DR progenitors following a fractionnated TBI (2x2Gy, 12h) at 24 h. Our preliminary results have shown an increase of CD34 cells and a specific decrease of CD 45 Ro cells which seems to be related to apoptosis.
Starting a single cell level study of DNA Damage Inducible genes has been considered as a priority for future development of specific new tools both for diagnosis and treatment of irradiated patients.PCR in situ hybridization was investigated. We expect to check for oncogenes (fos, jun) and other transcription factors (NFkB, C/EBP) as well as cytokines (TNF α, IL 1, βFGF, αINF). Our first feasibility tests for in situ PCR,included HIV specific primers hybridized with digoxygenin lbelled probes. In the serial dilution against human cell lines infected with HIV, the number of labelled cells was proportional to the cell dilution. No signal was observed without PCR amplification.

3-To extent the BMT indications (possibilities), an experimental study on the MHC-haploincompatible BMT in mice is going on. A specific allodepletion is performed with a mouse anti-IL2 receptor A chain-specific PC61-immunotoxin (PC61-IT). This immunotoxin strongly inhibited a primary mixed lymphocyte culture (MLC) and major histocompatibility complex (MHC)-restricted cytotoxicity. The allodepleted T cells retained their proliferative and cytotxic capacities in response to third-party

stimulation (cells H2 incompatible with donor and with recipient cells) demonstrating that PC61-IT specifically deleted recipient-antigen T cell clones from the donor mouse. The ability of this specific allodepletion to prevent graft-versus-host disease (GVHD) and graft rejection was investigated in vivo. Immunotoxin-depleted activated parental T lymphocytes (C3H/eB)were intravenously injected into lethally irradiated CDF1 mice. GVHD was evaluated after 6 days on the severity of gut lesions. PC61IT treated cells significantly reduced both donor T cell infiltration and acceleration of epithelial renewal (a sensitive index of gut damage) as compared to the corresponding untreated controls. The effect of selective allodepletion on prevention of GVHD and graft rejection was further studied after MHC-haploincompatible bone marrow transplantation. A significant increase in survival was observed in mice receiving $2x10^6$ T -depleted marrow cells and 0.5×10^6 PC61IT treated cells, since one third alive without GVHD (and with stable full or partial engraftment) after 100 days, whereas all the mice infused with bone marrow and sham-treated T cells (untreated T cells = positive control) died within 80 days from GVHD and all the mice infused with bone marrow alone rejected graft.
Furthermore, specific tolerance in chimeras towards donor cells could be demonstrated. These results as observed in an experimental in vivo model corroborate previous results obtained in vitro in humans and lead to consider the use of this selective allodepletion in human bone marrow transplant from donors other than identical familial siblings.

Papers published :
1 - Chaillet MP, Cosset JM, Pico JL et al, Prospective study of the clinical symptoms of therapeutic whole body irradiation. Health Phys. 64, 330, 1993.
2 - Cosset JM, Raffoux C, Chaillet MP, Socié G., ...Dubray B., Girinski T. Class I and 2 HLA typing after a 10 Gy irradiation. Health Phys. 64, 667, 1993.
3 - Delic J., Morange M., Magdelenat H. Ubiquitin Pathway involvment in human lymphocyte γ irradiation induced apoptosis. Molecular and Cellular Biology, 13, 4875, 1993
Papers submitted :
4 - Girinski T...Cosset JM, Socié G., Magdelenat H, Roger R. Peripheral blood corticotropin releasing factor (CRF), ACTH and cytokines (IL1b, IL6, TNFa) levels after high and low doses total body irradiation. *Lancet*
5 - Cavazzana-Calvo M. Stephan JL., Sarnacki S. et al, Attenuation of graft versus host disease and graft rejection by ex vivo immunotoxin elimination of alloreactive T cells in an H2 haplotype disparate mouse contamination, *Blood. (accepted)*
6 - Socié G., Cosset JM, Carosella E.....Gluckman E. Accidental protracted radiation induced aplastic anemia : clinico-biological report. *Blood*.
7 - Socié G., Gluckman E., et al. Bone marrow transplantation for Fanconi anemia using low dose cyclophosphamid/thoraco abdominal irradiation as conditional regimen : chimerism study by the polymerase chain reaction. *Blood (accepted).*

Progress Report

Contract: **FI3P-CT930069** **Sector: B21**

Title: Radiation effects and their treatment on the connective and vascular tissues in various organs.

1) Magdelenat CIR
2) Van der Kogel Univ. Nijmegen

I. Summary of Project Global Objectives and Achievements

The objective of the project is the development of new methods of diagnosis, prevention and treatment of alterations of connective or supportive and vascular tissues in various organs following radiation overexposure, based on an increased knowledge of physiopathological alterations. The organs or tissues considered are the lungs, muscles and brain.

Head of project 1 : Henri Magdelénat

II. Objectives for the reporting period :

The objectives of the reporting period (May 1993-August 1993) were:
-Initiation of a clinical trial for the evaluation of Superoxyde Dismutase in the treatment of radiation induced lung fibrosis.
-Initiation of an experimental protocol for the sequential study of Magnetic Resonance Imaging (MRI) of irradiated skeletal muscles in relation with histopathological features.
-Initiation of coordinated experimental protocols for the study of radiation induced (high dose) vascular alterations in the brain.
-Initiation of coordinated protocols for the study of behavioral alterations after irradiation of the brain (low or intermediate dose).

Progress achieved including publications

1 - Treatment of radiation-induced fibrosis of the lung by Superoxyde Dismutase (SOD)

Lung fibrosis affects a number of patients exposed to accidental or therapeutical ionizing radiations on the thorax
A pilot study has been conducted on 9 patients presenting with symptomatic lung fibrosis which developped 5 to 9 months after radiotherapy. Bovine SOD was administered by inhalation (aerosols) : 35mg (125 000 units) 3 times a week (every second day) for 2 weeks (Total SOD required : 210mg). Based on an estimated aerosol deposition of 10% in the lung, the SOD delivered to the lung was approximately 75000 units.
Cough symptoms improved in 9/9 patients, breathing frequency in 8/9 with an average gain in peak flow of 34 % .Thorax X ray showed objective response in 6 patients. No toxicity was observed.
The programme, once interrupted due to recent restrictions on the use of bovine proteins for clinical investigations, will now resume under the form of a controlled phase II clinical protocol with an authorized source of bovine SOD.

2 - Experimental sequential study of Magnetic Resonance Imaging (MRI) of irradiated skeletal muscles in relation with histopathological features.

Twenty two New-Zealand rabbits have been irradiated (Dr Lefaix, Jouy en Josas) at 120 Gy with a source of Iridium 192, then examined regularly (twice a month) by 1.5 Tesla proton magnetic resonance imaging (MRI), and séquentially biopsied to study the histological evolution from early inflammatory reaction to installed fibrosis and the alterations of the prolin/hydroxyprolin ratio of fibrotic areas. At 8 weeks, a grade 1 fibrosis appeared, after a period characterized by the presence of inflammatory cells, increase of oedema in the dermis and microvascularization in the underlying muscular tissue.MRI (T1, T2 or Turbo-flash) was modified as soon as the second week after irradiation, indicating oedematous and vascular changes. The collection of sequential MRI data, tissue samples and histochemical data continues.

3 - Cranial radiation induced dementia

In order to develop a model of radiation-induced behavioral dysfunction, a course of whole brain radiation therapy (RT, 30 Gy/10 fractions/14 days) was administered to 26 Wistar rats aged 16-27 months while 26 control rats received sham irradiation. Sequential behavioral studies including one-way avoidance, two-way avoidance and a standard operant conditioning method were undertaken. In addition, rats were studied in a water maze 7 months post-RT.

-One-way avoidance

Prior to RT, both groups were similar reaching 70% of correct response, ie avoidance, at the 5th daily session. One month post-RT (first recall), the mean percentage of avoidance decreased uniformly in control and irradiated rats. Three months post-RT, the results of the control group were stable while the results of the RT group began to drop. At 6 months post RT (third recall), irradiated rats had a much lower percentage of avoidance (23%+-3.9) that controls (55%+-7.65) ($p<0.001$).

Similarly two-way avoidance test, press lever avoidance test and water maze test showed behavioral dysfunction for the irradiated group versus control.

This is the first study demonstrating behavioral dysfunction following a course of conventional RT in an experimental model. It is likely that the old age of the rats at the date of RT is a predisposing factor for RT-induced behavioral dysfunction. This model can be used to study the pathogenesis of radiation-induced dementia.

III. Objectives for next period :

- Treatment and evaluation of 30 patients with lng fibrosis by SOD

- Sequential magnetic resonance imaging (MRI)semeiology of radiation-induced fibrosis to be correlated with objective histological grading and biochemical analysis of proline / hydroxyproline ratio.

Participating scientists

Benyahia B	(Institut Curie)
Campana F	(Institut Curie)
Dao T H	(Institut Curie)
Lefaix J L	(C.E.A.)
Magdelénat H	(Institut Curie)
Perdereau B	(Institut Curie)
Rahmouni A	(C.H.U. Créteil)

Head of project 2 : Pr. A.J. Van der Kogel

II. Objectives for the reporting period :

In this part of the project work is focused on radiation injury of the central nervous system, in particular on the role of connective (glial cells and their precursors) and vascular (endothelial cells) tissue elements and their interactions.

Progress achieved including publications

During the first part of the project, the following has been done in relation to the topics described in the initial application :

1 - Pathophysiology of supporting cells in the nervous sytem : the role of growth factors/cytokines and oxygen-radical scavenging mechanisms in radiation injury in astrocytes, oligodendrocytes and glial progenitor cells.

In addition to the use of a clonogenic assay for glial progenitors, a quantitative proliferation assay has been developed to assess the effects of growth factors and cytokines on the radiation response of glial progenitor cells. The scoring of fluorescently stained colonies can now be performed with the aid of a computerized image-analysis sytem.

In contrast to the clonogenic assay for glial progenitors which uses a monolayer of astrocytes as a feeder layer, in this assay no feeder layer (with an endogenous production of cytokines and GF's) can be used. Therefore various substrates have been tested with varying success. We have now settled on poly-Lysine coated multi-well culture plates, using ^{3}H-TdR incorporation at various times after treatment as a measure of proliferation and indirectly of cell survival. At this moment bFGF and PDGF have been tested in combination with irradiation, showing a significant enhancement of surviving cells after bFGF and to a lesser extent after PDGF. The timing of administration has been varied, as well as the concentration of GF's and, and complete dose-survival curves have been obtained.

2 - Clinical studies : non-invasive (MRI and MRS) evaluation of brain injury after radiotherapy in combination with administration of nicotinamide and carbogen breathing.

This new approach to brain tumor treatment has recently been started in Nijmegen University Hospital as an attempt to overcome tumor hypoxia by relatively non-toxic additives of radiotherapy. Since we have seen an enhanced toxicity of normal nervous tissue in animal studies, we have initiated clinical follow-up studies with MRI and ^{1}H- & ^{31}P-MR spectroscopy to detect tumor response and possibly changes in normal brain.

At this moment 14 patients have been entered in this protocol, combining radiation treatment with nicotinamide and carbogen breathing. ^{1}H-MRS was carried out in 9 patients, showing up till now no significant changes. However, it is thought that the measurement of ^{31}P-spectra will yield better information on changes in the energy metabolism, as indicated in the (preliminary) results obtained for one patient.

III. Objectives for next period :

1a. In vitro experimental studies

Studies on the effects of bFGF, PDGF, TNF, and interleukins on the radiation response of various classes of glial cells (astrocytes, oligodendrocytes, glial progenitors) will be continued. In the near future the role of SOD and other potential modifiers of oxydative injury will be further investigated.

The development of other *in vitro* models of cellular interactions is actively pursued in the laboratory in Nijmegen. In particular, the role of TNFα (produced by astrocytes) in cellular necrosis and changes in endothelial permeability.

In close collaboration with dr. Magdelenat (Institut Curie), we will try to correlate the effects observed for various GF's and cytokines with their receptor expression. Also, the project is aimed at elucidating the role of different modes of cell death (mitotic and apoptotic) in specific glial cell populations, as preliminary work has shown large differences in the relative contributions ot these types of cell death after irradiation.

1b. In vivo experimental study

A method has been developed to study vascular perfusion and permeability in gliomas and the normal rat brain after irradiation. A comparison will be made of results obtained with quantitative morphology (image analysis of vascular architecture, perfusion, and permeability) and results obtained with NMR spectroscopy and imaging (D_2O and Gd-DTPA perfusion/diffusion).

In collaboration with the Institut Curie, we will quantitative the vascular architecture of the brain of aging rats at long times after irradiation by immunocytochemical staining followed by image analysis.

Along the lines of the current project, animal studies on the possible modification of the development of radiation injury by SOD, ACE-inhibitors (Captopril) and inhibitors of polyamine synthesis (DFMO) have been started in the rat spinal cord.

2. Clinical studies

NMR spectroscopy and imaging are now being used to evaluate the effect of nicotinamide combined with carbogen breathing and radiotherapy on brain tumors and normal brain tissue. In patients dynamic Gd-DTPA imaging is used to measure vascular perfusion/diffusion, and combined with ^{1}H- & ^{31}P- MRS to evaluate metabolic changes. A double tuned measuring coil is being built for this procedure.

Progress Report

Contract: **FI3P-CT920064b** **Sector: B22**

Title: Reduction of risk of late effects from incorporated radionuclides.

1)	Stradling	NRPB
2)	Volf	KFK
3)	Poncy	CEA - Bruyères-le-Châtel
4)	Archimbaud	CEA - Pierrelatte
5)	Burgada	ADFAC

I. Summary of Project Global Objectives and Achievements

The chelating agents of choice for enhancing the excretion of transportable forms of Pu, Am and Th from the human body are the trisodium calcium and -zinc salts of diethylenetriaminepentaacetic acid (DTPA). These substances are not completely effective and previous studies by the partners in collaboration with the Univ. of California and Lawrence Berkeley Laboratory have shown that the analogues of siderophores, notably the hydroxypyridinone derivative code named 3,4,3-LIHOPO is appreciably more effective than DTPA after the inhalation and intravenous injection of Pu; the ligands were considered equally effective for Am under these conditions.

The objectives of the further studies summarised in this report were

(1) to investigate the comparative efficacies of 3,4,3-LIHOPO and DTPA for Pu, Am and Th in the rat after simulating wound contamination by subcutaneous and intramuscular injection (01)

(2) to investigate the efficacies of 3,4,3-LIHOPO and two other siderophore analogues DFO-HOPO and DTPA-DX for intravenously injected Pu and Am when administered by injection, gavage or infusion (02)

(3) to optimise treatment for inhaled Pu-tributylphosphate using 3,4,3-LIHOPO (03)

(4) to commence detailed studies on the toxicology of 3,4,3-LIHOPO (03).

The 3,4,3-LIHOPO used for these studies was synthesised at ADFAC (05). The other analogues were supplied by the Univ. of California. The results of this work have shown that, potentially, 3,4,3-LIHOPO represents a most significant advance for the decorporation of Pu, Am and Th. Preliminary toxicological data are encouraging in so far that analysis of blood and urine appear to be normal and that biopsies of liver and kidney do not show any significant histological alterations.

Other studies have been devoted to removal of uranium from the body for which no satisfactory substances are presently available. The substances examined here included new phosphonate complexes (04) synthesised at ADFAC (05) and calixarene complexes synthesised at Pierrelatte (04). Whilst the administration of some of these substances resulted in a significant increase in uranium excretion there is considerable room for improvement.

Head of project 1: Dr. Stradling

II. Objectives for the reporting period

The objectives of the reporting period were

(1) to compare the efficacies of 3,4,3-LIHOPO and DTPA for removing Pu and Am from the rat after simulated wound contamination taking due account of the treatment regimen, the delay in administration and mass deposited

(2) to commence studies on the comparative efficacy of 3,4,3-LIHOPO and DTPA for removing Th from the rat after simulated wound contamination

(3) to examine the efficacy of new diphosphonate compounds for enhancing the excretion of U.

The aims of the next reporting period are

(1) to complete the studies on Th after simulated wound contamination

(2) to examine the efficacy of 3,4,3-LIHOPO for inhaled Th

(3) to investigate the efficacy of inhaled 3,4,3-LIHOPO for Pu, Am and Th

(4) to continue the testing of new substances for decorporating U

(5) to improve the treatment for inhaled Pu and Am by the administration of DTPA in drinking water as reported previously.

III. Progress achieved including publications

A likely route of accidental intake of Pu and Am by workers is from wound contamination. With DTPA as a comparison, 3,4,3-LIHOPO has been examined for its ability to remove ^{238}Pu and ^{241}Am from the rat after the subcutaneous and intramuscular injection of 200 Bq of each actinide (0.3 ng Pu, 1.6 ng Am) administered as nitrate. For the subcutaneous experiments ^{238}Pu and ^{241}Am were injected into the region immediately above the extensor cruris muscle of the hind-leg. Intramuscular injections were at a depth of 5 mm at the centre of the extensor cruris muscle block.

After the subcutaneous injection of ^{238}Pu and ^{241}Am, both ligands were more effective after local administration than after intraperitoneal injection, intravenous injection or continuous infusion using an implanted osmotic pump. Dosages of 3 μmol kg^{-1} (d^{-1} by infusion) of 3,4,3-LIHOPO were more effective than dosages of 30 μmol kg^{-1} DTPA (d^{-1} by infusion) after each mode of administration.

The most effective regimen of those investigated for subcutaneous ^{238}Pu and ^{241}Am involved the local administration of 30 μmol kg^{-1} of 3,4,3-LIHOPO 30 min after exposure followed by intraperitoneal injections of 30 μmol kg^{-1} at 6h, 1, 2 and 3d. By 7d, the amounts of ^{238}Pu and ^{241}Am in the body, ie. wound site and other tissues, were 2% and 7% of those in controls. When DTPA was administered under these conditions, the corresponding values were 20% and 26%.

The ligand 3,4,3-LIHOPO was even more effective for ^{238}Pu and ^{241}Am after their intramuscular injection. After a single local administration of 30 μmol kg^{-1} at 30 min, the amounts retained in the body by

7d were respectively 0.9% and 0.8% of those in controls. The corresponding values after the local and repeated intraperitoneal administration of 30 μmol kg^{-1} DTPA were 32% and 22%. The increased efficacy of 3,4,3-LIHOPO after intramuscular injection was attributed to the greater retention of ^{238}Pu and ^{241}Am at the wound site (98% v 67% of the initial deposit) or the commencement of treatment.

An important consideration is the delay between exposure and treatment. The efficacy of both ligands was reduced appreciably with time although 3,4,3-LIHOPO retained its superiority. For example when repeated treatment with 30 μmol kg^{-1} of 3,4,3-LIHOPO commenced 6h and 1d after the intramuscular injection of ^{238}Pu the amounts retained in the body 7d after exposure were respectively 8% and 19% of those in controls; the corresponding values using DTPA were 66% and 75%. The data for ^{241}Am were similar to those for ^{238}Pu. These results have implications for the treatment of humans with DTPA. If administration is delayed, until, for example, more information on the severity of the accident is available, treatment is likely to be only partially effective.

Another consideration is the effect of mass deposited at the wound site. When the amount of Pu (180 ng as ^{239}Pu) was increased 600 fold the efficacy of treatment decreased. After the local administration of 30 μmol kg^{-1} 3,4,3-LIHOPO at 30 min and intraperitoneal injections at 6h, 1, 2 and 3d after exposure the amounts of ^{239}Pu retained in the body 7d after subcutaneous and intramuscular injection were respectively 7% (cf 2%) and 5% (cf 0.9%) of control values. The corresponding amounts after DTPA administration were 33% and 47%.

The results above show that 3,4,3-LIHOPO represents potentially a most significant advance for the treatment of wounds contaminated by Pu and Am. Detailed toxicity of the ligand is being undertaken at Bruyeres-le-Chatel and Fontenay-aux-Roses.

The ligand DTPA is also the substance of choice for thorium. Preliminary studies have been undertaken on the comparative efficacy of 3,4,3-LIHOPO and DTPA in the rat after the subcutaneous injection of 300 Bq of ^{228}Th as nitrate. When 30 μmol kg^{-1} of 3,4,3-LIHOPO were administered locally 30 min after exposure the amounts of ^{228}Th retained at the wound site and systemic tissues were reduced to 20% of controls by 7d. After the repeated administration of 30 μmol kg^{-1} DTPA using the regimen given previously the corresponding values were 94% and 75%. The 4.5 fold greater reduction in the total body content with the administration of 3,4,3-LIHOPO was similar to that achieved after the alveolar deposition of ^{234}Th reported previously. More detailed studies are in progress which are designed to optimise treatment with 3,4,3-LIHOPO and to examine the influence of the delay in treatment and mass of Th deposited at the simulated wound sites.

The examination of three diphosphonate derivatives received from ADFAC (05) is in progress but no experimental results are available at present.

Publications

1. Stradling GN, Gray SA, Ellender M, Moody JC, Hodgson A, Pearce M, Wilson I, Burgada R, Bailly T, Leroux YGP, Manouni D El, Raymond KN and Durbin PW. The efficacies of 3,4,3-LIHOPO and DTPA for enhancing the excretion of plutonium and americium from the rat: comparison with other siderophore analogues. Int. J. Radiat. Biol. 62, 487-497, 1992.

2. Stradling GN (ed). Reduction of Risk of Late Effects from Incorporated Radionuclides. EULEP Newsletter 72, 3-18, 1993.

3. Stradling GN, Gray SA, Moody JC, Pearce MJ and Wilson I. Efficacy of 3,4,3-LIHOPO for enhancing the excretion of plutonium and americium from the rat after simulated wound contamination as nitrates: comparison with DTPA. Memorandum-M383, Chilton: NRPB, January 1993.

4. Stradling GN, Gray SA, Ellender M, Pearce M, Wilson I, Moody JC and Hodgson A. Removal of inhaled plutonium and americium from the rat by administration of DTPA in drinking water. Human Expt. Toxicol. 12, 233-239, 1993.

5. Stradling GN, Gray SA, Moody JC, Pearce MJ, Wilson I, Burgada R, Bailly T, Leroux Y, Raymond KN and Durbin PW. The efficacies of 3,4,3-LIHOPO and DTPA for enhancing the excretion of plutonium and americium from the rat after simulated wound contamination as nitrates. Int. J. Radiat. Biol. 64, 133-140, 1993.

6. Stradling GN, Gray SA, Moody JC, Wilson I, Pearce MJ, Burgada R and Raymond KN. Comparative efficacy of 3,4,3-LIHOPO and DTPA for plutonium (IV) and americium (III) after simulated wound contamination. Proc. of 4th International Symposium on Chelating Agents in Pharmacology, Toxicology and Therapeutics, Pilsen, August 1993. To be published in Plzensky Lekarsky Sbornik (Plzen Medical Report).

7. Stradling GN. Recent progress in decorporation of plutonium, americium and thorium. Proceedings of Workshop on Intakes of Radionuclides, Bath, September 1993. To be published in Radiat. Prot. Dosim.

8. Gray SA, Stradling GN, Pearce MJ, Wilson I, Moody JC, Burgada R, Durbin PW and Raymond KN. Removal of plutonium and americium from the rat after simulated wound contamination with 3,4,3-LIHOPO and DTPA : Effect of delayed administration and mass of plutonium. ibid.

Head of Project 2: Prof. Volf

II. Objectives for the reporting period

To perform final experiments and evaluate the data on decorporation of intravenously or intramuscularly injected Pu-238, Am-241 and Th-234 in rats, especially with DFO-HOPO and 3,4,3-LIHOPO.

To perform final evaluation of the experiment on reduction of bone tumour risk after incorporation of Pu-239. Histopathological examination of rat tissues should have been performed by Prof. Luz (Institute of Pathology, GSF Neuherberg).

III. Progress achieved including publications

A simulated delayed protracted treatment of actinides originally deposited in a contaminated wound was carried out on rats.

Results of experiments with DFO-HOPO and 3,4,3-LIHOPO after i.v. injection into rats were partly published but the dose-effect and time-effect relationships, when the chelators are administered in s.c. injections or by oral gavage have to be prepared for publication.

Final evaluation of the experiment on reduction of bone tumour risk after incorporation of Pu-239 in rats will be only possible if all data of histopathological examination of dissected tissues will be available. This depends upon the Institute of Pathology, GSF Neuherberg.

A simulated delayed protracted treatment of actinides originally deposited in a contaminated wound was carried out on rats by administration of 3,4,3-LI-HOPO after single i.m. injection of Pu-238 and Am-241.

Treatment was initiated by a single local injection of 3,4,3-LIHOPO at 4d or 30d after injection of actinides. At the same time, diffusion minipumps were implanted s.c., providing continuous infusion of the above chelator for a further 4 weeks.

In untreated rats, about 95% of injected actinides were translocated from the injection site after 60d. Total retention of Pu-238 and Am-241 in the bones and liver of these animals was 45% and 38% of injected dose, respectively.

Whole body counting of Am-241 indicated that a substantial fraction can be removed by the above treatment, even if it was initiated as late as 30d after injection of Am-241. This effect was further characterized by determination of contents of the actinides at the injection site and in dissected organs. First, treatment with 3,4,3-LIHOPO did not further mobilize actinides at the injection site as observed 60d after their injection. Second, organ retention of activity was reduced to about 20% and 60% of control values when treatment started at 4d and 30d after injection of actinides, respectively.

Publication

V. Volf, R. Burgada , K.N. Raymond and P.W. Durbin, Early chelation therapy for injected Pu-238 and Am-241 in the rat: comparison of 3,4,3-LIHOPO, DFO-HOP, DTPA-DX, DTPA and DFOA. International Journal of Radiation Biology, 1993, vol. 63, 785-793.

OBJECTIVES FOR THE REPORTING PERIOD

To perform final experiments and evaluate the data on decorporation of intravenously or intramuscularly injected Pu-238, Am-241 and Th-234 in rats, especially with DFO-HOPO and 3,4,3-LIHOPO.

To perform final evaluation of the experiment on reduction of bone tumour risk after incorporation of Pu-239 in rats. Histopathological examination of tissues should have been performed by Prof. Luz (Institute of Pathology, GSF Neuherberg).

OBJECTIVES FOR THE NEXT YEAR

Detailed results of experiments with DFO-HOPO and 3,4,3-LIHOPO after intravenous injection of Pu-238 and Am-241 into rats, especially those on dose-effect and time-effect relationships, when the chelators are administered in s.c. injections or by oral gavage, will be prepared for publication.

Final evaluation of the experiment on reduction of bone tumour risk after incorporation of Pu-239 in rats will be possible only if all data of histopathological examination of dissected tissues will be available.

Head of Project 3: Drs J-L. Poncy/F. Pacquet

II. Objectives for the reporting period

To compare the efficacy of 3,4,3-LIHOPO and DTPA for removing plutonium inhaled by Sprague-Dawley rats as the tributylphosphate complex.

To study the toxicology of 3,4,3-LIHOPO, on non-human primates.

III. Progress achieved including publications

The potential toxicity of 3,4,3-LIHOPO was investigated in baboons with:

1) clinical laboratory measurements used for humans
2) observations of histological changes in biopsies of baboon kidneys and livers.

Table: Characteristics of baboons (Papio papio) used in this study

No. monkey (sex)	Date of birth	Treatment	Weight 3.6.92
1st experiment: Treatment June/July 1992			
523 (f)	22.7.88	control	4.6 kg
529 (m)	11.8.89	control	5.6 kg
534 (m)	26.7.90	control	3.7 kg
530 (m)	8.10.89	DTPA (30µmol./kg)	4.8 kg
531 (m)	20.12.89	DTPA (")	4.7 kg
535 (m)	25.10.90	LIHOPO (30 µmol./kg)	3.1 kg
2nd experiment: Treatment April/May 1993			**27.4.93**
529 (m)	11.8.89	DTPA (30µmol./kg)	8.3 kg
534 (m)	26.7.90	LIHOPO (3µmol./kg)	5.8 kg
536 (m)	4.3.91	LIHOPO (30µmol./kg)	3.6 kg

Chelating agents were injected at dosages of 30 or 3µmol kg^{-1}, intravenously on day 0 and intramuscularly on days 3,6,9,13,16,20,23 and 26. Urine and blood samples have been collected just before, at the end and 6 months after the end of chelate treatments. Parameters which have been analysed, were in serum: glucose, nitrogen compounds, electrolytes, enzymes, total proteins and lipids, in urine: diuresis, glucose, nitrogen compounds and electrolytes. The different biochemical parameters observed early and later after the end of 3,4,3-LIHOPO treatment did not show significant changes which might traduce kidney and hepatic disturbances.

Biopsies of liver and kidneys were performed on each animal one week after the end of chelate administrations and others were planned 6 months later. Observations in light microscopy on semi-thin sections did not show significant structure alterations in hepatic and renal tissues from treated animals by either 3,4,3-LIHOPO or DTPA. Only few vacuoles were observed in hepatic cells after LIHOPO administration. The electron microscopy study confirm the minor toxicity of 3,4,3-LIHOPO just after treatment which does not inhibit the recovery of the cells.

In another way, experiments were done to define the best dosage for 3,4,3-LIHOPO, to reduce the plutonium contamination. Previous studies (Poncy et al, 1993) used the ligand at a dosage of 30µmol/kg of body weight and have shown significant reduction of the radionuclide level. When compared to the DTPA, the 3,4,3-LIHOPO seemed to be more efficient, and this efficacy was independent of the inhaled mass of plutonium. Recent studies (Pacquet et al, 1993) tried to assess the efficiency of the siderophore analogue when used at lower dosage. Experiments with 3,4,3-LIHOPO used at a dosage of 3µmol/kg of body weight gave some conclusions:

1) The 3,4,3-LIHOPO was very effective to decorporate plutonium when used at low dosage (3µmol/kg).

2) The efficacy of the ligand was, in some cases, higher than that of DTPA 30µmol/kg.

3) The efficacy of LIHOPO "3µmol/kg" seem to be dependent of the inhaled mass of plutonium.

4) The importance of very prompt treatment after contamination was pointed out.

Objectives for the next year

The potential toxicity of the 3,4,3-LIHOPO will be investigated with new experiments with baboons and studies of morphological changes in electron microscopy.

Other experiments will be done with rodents to evaluate the efficacy of the ligand to decorporate other transuranic elements. The neptunium is currently used in the nuclear industry and no effective treatment is available, since DTPA has a very limited action.

Publications

Poncy J-L, Rateau G, Burgada R, Bailly T, Leroux Y, Raymond KN and Masse R. The efficacy of 3,4,3-LIHOPO for reducing the retention of ^{238}Pu in rats after inhalation as the tributyl-phosphate complex. Int. J. Radiat. Biol. (in press).

Pacquet F, Poncy J-L, Rateau G, Burgada R, Bailly T, Leroux Y, Raymond KN and Masse R. Reduction of the retention of ^{238}Pu inhaled as the tributyl-phosphate complex in rats treated with 3,4,3-LIHOPO. To be presented in congress "Intakes of radionuclides" 13-17 September 1993, Bath, United Kingdom.

Head of Project 4: Dr. Archimbaud

II. Objectives for the reporting period

To test new substances for enhancing the excretion of uranium from the body. To investigate the toxicity of calixarene complexes.

III. Progress achieved including publications

DECORPORATION STUDIES

Tested molecules

Cyclohexane diamine tetramethane phosphonic acid - CDTPA
Ethylene diamine tetramethane phosphonic acid - EDTP
Calix(8)arene without terbutyl - S8(D)

Methods

The animals used were Sprague Dawley rats weighing about 200g. They were housed in metabolism cages. To assess the decorporation effectiveness in vivo of the different molecules, the animals were first intoxicated by intramuscular injection with 1ml of a solution of uranyl nitrate 2.10-5M added to physiological serum at pH=6. The injected quantity was 200µg/kg. The calixarene was mixed in oil because it was liposoluble. The chelating agents were injected intraperitoneally half an hour after intoxication. The ratio of chelating molecules to uranyl molecules is equal to 100. For each chelating molecule a control batch and a batch of animals injected with the decorporating agent were killed 48 hours after intoxication. For each animal, uranium concentration was analysed in the kidney, bone and urine.

Results

The results are shown in Table 1.

	Percentage / Control			
	Vol. urine	U.urine	U.kidney	U.bone
EDTP + Dimazon	88	80		
CDTPA	50	74*	35	130
S8 (D)	56	84	135	182

*Significance level 5%.

Discussion

We observed that diuresis was decreased by applying EDTP and CDTPA. So we treated the animals with EDTP and a diuretic. The uranium excretion is similar to controls. With the CDTPA and calix(8)arene (D) we noted a uranium increase in the bone.

Study of interference between sulphonate calix(8)arene (S8) and the metabolism of copper

We observed on treated animals with S8 some signs comparable to Wilson disease (anorexia, hemolytic anaemia, icterus, haemoglobinury). So we have verified if the presence of S8 induced a copper increase in the liver. Otherwise, if a deficiency of copper in food decrease the calixarene toxicity.

Methods

The animals used were Sprague Dawley rats weighing about 200g. They were housed in metabolism cages. Ten days before the injection of S8 they were fed with a food without copper. The normal food concentration in copper is 600mg/kg. We have injected 31mg/kg and 50mg/kg of S8. The animals were killed 7 and 6 days after the injection. Every day each animal was weighed. The copper in the liver was analysed after mineralisation in a microwave oven by atomic absorption.

Results

Table 2 indicates the variation of weight and the concentration of copper in the liver.

Controls			Injected		
Weight (g) variation	mgCu/ liver	µgCu/ g.liver	Weight variation	mgCu/ liver	µgCu/ g.liver
+25	72	2.9	-40	52	3.3
+40	72	2.7	-40	76	3.3
+40	70	2.9	-49	72	4.0
+35	68	3.3	-28	78	3.2
+22	74	2.7	-27	86	4.0
			-36	151	6.2
			-37	119	4.6

Discussion

This study shows that calixarene allows the reconcentration of copper in the liver but it does not explain the total toxicity of this molecule. In fact the animals fed without copper showed an important loss of weight while the concentration of copper in mg/g liver did not increase. Otherwise the copper concentration in liver of some controls was comparable to those of intoxicated animals. Sulphonate calix(8)arene could chelate other oligo-element metals. This hypothesis can explain its toxicity and its non efficiency as decorporating agent.

For the next year we will continue screening about different molecules synthesised by French laboratories. For calixarene we will mark the S8 with C14 to understand its metabolism and we will try to synthesise the calix(12)arene. The efficiency of a chelating agent depends on the stability of the complex formed with the contaminant in the different biological conditions. In the case of the contaminants urinary excreted, the conditions are quite different when the complex leaves the blood to enter in the kidneys. We will try to check a method to study the stability of a complex between a chelating agent and a contaminant in the conditions encountered in the kidneys.

Head of project 5: Dr. Burgada

II. Objectives for the reporting period

(a) To Synthesize the siderophore analogue 3,4,3-LIHOPO so that its efficacy for removing Pu,Am and Th from experimental animals could be investigated by CEA, Kfk and NRPB.

(b) To synthesize new phosphonic acid derivatives for use by the CEA and NRPB in studies designed to remove uranium from the body.

III. Progress achieved including publications

3.15 g. of **3-4-3 LIHOPO** was synthesized and distributed amongst the partners as follow :

Dr.G.N.Stradling (NRPB) 2g. December 1992- April 1993

Dr.J.L.Poncy (CEA) 1g. April 1993.

Pr.Dr. V.Volf (KFK) 150 mg. July 1993

Phosphonates. Distribution amongst the partners:

1g. of NTP and 1 g. of DETPP was synthesized for Mrs. Archimbaud (CEA-Pierrelatte) October 1992.

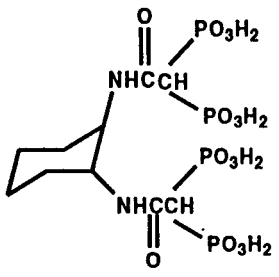
1 g. of NTP , 1 g. of DETPP and 1 g. of CDTT for Mrs. Archimbaud March 1993.

500 mg. of CDTT, 500 mg. of CDMT and 500 mg. of XCMD for Dr. G.N.Stradling April 1993

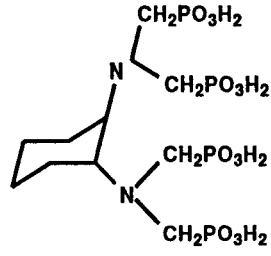
1-2 diamino cyclohexyl tetrametylene tetraphosphonate (CDTT)

1-2 diamido cyclohexyl methane tetraphosphonate (CDMT)

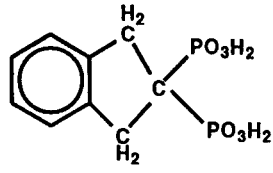
1-2 cyclomethane xylil bis phosphonate (XCMDP)



CDMT

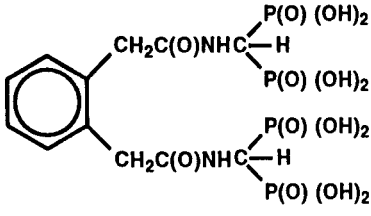


CDTT

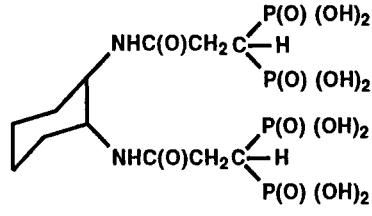


XCMDP

Two new compounds have been prepared DDMT and CDET but not yet submitted to the partners :



DDMT



CDET

We are waiting for the results of biological tests of decorporation to put forward the synthesis of new diphosphonates. The comparison between CDET, CDMT and CDTT is of particular interest in relation with their acido-basic properties and steric factors. For example the two phosphonics groups are separated by three atoms in CDTT and only by one in CDET and CDMT. This lead to a different size of the interaction of the two chelating groups in the space. On another way CDTT presents two basics' groups (the nitrogen atom) inversely there is not the case in CDET and CDMT where the nitrogen atom is amidic.

8) List of publications:

The efficacies of 3,4,3-LIHOPO and DTPA for enhancing the excretion of plutonium and americium from the rat:comparison with other siderophores analogues

Int.J;Radiat.Biol.,1992,vol.62,n°4 p.487.

GN;Stradling,S.A.Gray, M.Ellender, J.C.Moody,H.Hodgson, MPierce, I.Willson, R.Burgada, T.Bailly, Y.Leroux,D.El Manouni, K.N.Raymond and P.W.Durbin.

Early chelation therapy for injected Pu 238 and Am 241 in rats. Comparison of 3,4,3 LIHOPO, DFO-HOPO, DTPA-DX, DTPA and DFOA.

Int.J;Radiat.Biol., Accepted for publication.

V.Volf, R.Burgada, K.N.Raymond and P.W.Durbin

The efficacies of 3,4,3-LIHOPO and DTPA for enhancing the excretion of plutonium and americium from the rat after simulated wound contamination as nitrates.

Int.J;Radiat.Biol., Accepted for publication.

GN;Stradling,S.A.Gray,J.C.Moody,H.Hodgson,M.Pierce,I.Willson,R.Burgada,T.Bailly,Y.Leroux,K.N.Raymond and P.W.Durbin.

Progress Report

Contract: **FI3P-CT920059** **Sector: B23**

Title: Radiation effects and their treatment after local exposure of skin and sub-cutaneous tissues.

1) Masse	CEA-FAR
2) Hopewell	Univ. Oxford
3) Coggle	Hosp. St. Bartholomew
4) Di Carlo	IFO

I. Summary of Project Global Objectives and Achievements

Skin irradiation by combined β and total γ / X irradiations, which occurred in Chernobyl and Goiana accidents provoked lesions poorly documented in man up to now. Experimental approach of these problems were performed in pigs and mice to test new diagnosis and prognosis methods, to study the pathogenesis and to propose treatments. On the other hand, clinical assays were carried out in man with regards to diagnosis and treatments of radiation-induced late effects on skin.

In pigs, a species whose skin is the most similar to human skin, the experiments were worked out using 90Sr-90Y β-irradiation (32 - 80 Gy), combined or not with γ-irradiation with 192Ir at 10% of the β dose. NMR imaging of the irradiated pig skin permitted diagnosis as early as 32 h and at a dose as low as 32 Gy; skin blood flow modifications made obvious the different stages of the skin reaction: hyperemia (vasodilatation) and ischemia (thrombosis of the superficial vessels) (*Saclay*).

In mice, total X-irradiation (4, 6 and 8 Gy) had no effect on time of onset of skin reactions (local 50 Gy β-irradiation) but the plateau's and the final resolution of the lesions were significantly prolonged (*London*).

In pig, cellular and molecular characterization of irradiated skin fibroblasts showed early changes in the expression of proto-oncogenes c-jun and c-fos, in relation with sampling time and dose (*Saclay*).

In mice TGF-β expression and TNF-α levels were significantly different after skin irradiation between remaining scar tissue and dermally derived tumors (*London*).

Early and delayed effects prevention and treatment in pigs were performed using oral administration of essential fatty acids before and after irradiation: a significant modification could be pointed out concerning first erythema, moist desquamation, mauve reaction and dermal necrosis (*Oxford*).

Pigs were exposed to high doses of γ rays in order to obtain a large and permanent cutaneo-muscular fibrosis and they were treated with parenteral injections of liposome encapsulated bovine SOD 6 months after exposition: the reduction of the fibrosis volume was highly significant (*Saclay*).

In radiation workers diagnosis of late vascular effects, specially in the hands, thermography (thermal recovery after thermostimulation) was a very usefull tool for the following of patients without any patent clinical radiation damage; histological and ultrastructural examination of skin samples were relevant with biophysical diagnosis methods (*Paris and Rome*).

I. Head of project N°1: R. MASSE

II. Objectives for the reporting period

The first objective was to validate an experimental model of combined irradiation ($\beta + \gamma$) in pigs, easily reproducible and exploring a relatively wide range of dose. The second point concerned new non invasive methods of diagnosis, especially NMR imaging and spectroscopy of the skin, early after irradiation. Early modifications of cytokines and proto-oncogenes expression in fibroblasts irradiated *in vivo* and *in vitro* were studied in relation with samples timing and dose.

In patients suffering sub-cutaneous radiofibrosis attempts were made to compare histology and immunocytochemistry results with the beneficial effect of superoxide dismutase (SOD) topical treatment. Systemic treatment of radiation-induced cutaneo-muscular fibrosis in pigs with SOD seemed still faster and more efficient.

The close similarity between human and porcine skin radiation pathology might permit a soon application of these progress in managing of skin local overexposure in man.

III. Progress achieved including publications

Most of the available information in human deals with skin response after high dose irradiations from a collimated photon or electron source, or from uniform beta-emitting plaques. In accidental irradiation such uniform and well delimited exposures are rarely seen, and an experimental approach is essential. Consequently, the early and late non-stochastic effects of radiation have been studied in pigs, which is unanimously considered as the best experimental model for skin radiopathology.

1) Pathogenesis of radiation damage to skin

Pig skin has been exposed to β particles (local irradiation) with $^{90}Sr/^{90}Y$, combined with graduated doses of γ rays (^{192}Ir) given on a larger area (regional irradiation): 6 animals in each group were irradiated on the flanks with doses between 32 and 80 Gy on surface areas between 2 and 4 cm in diameter. The follow up of the lesions was performed clinically and using IR thermography and superficial blood flow measurements. NMR imaging was tested on 3 pigs irradiated each with 32, 64 and 96 Gy: T2 weighted spin echo images showed that irradiated areas signal intensities were significantly decreased during the three weeks following irradiation. The signal evolution was similar in skin, subcutaneous adipose tissue and skeletal striated muscle, but with noticely different amplitude. The larger variation was observed on skin.

In human skin *in vivo* γ-irradiated, the immunohistochemical studies demonstrated that dermal fibroblasts exhibited an activated "myofibroblastic" phenotype characterized by the expression of smooth muscle cells-α-actin microfilaments. *In vivo* and *in vitro* irradiated dermal fibroblasts overexpressed intranuclear Transforming Growth Factor β (TGF-β) compared to normal cells as intermediary epidermal cells irradiated in vivo. Platelet Derived Growth Factor Receptor-β (PDGF Rβ) are overexpressed in dermal fibroblasts of irradiated skin and Epidermal Growth Factor Receptors (EGFR) were overexpressed by all the cellular layers, many years after radiotherapy and appeared as pathognomonic indicators of previous irradiation.

2) Fibrosis treatment

Twelve pigs irradiated on the outer side of the thigh were exposed to 160 Gy of γ-rays in order to obtain a large and permanent cutaneo-muscular fibrosis; they were treated (intra-muscular injections) with liposome encapsulated SOD (native Cu-Zn bovine SOD) 6 months after exposition: its efficacy was highly significant, with a regression higher than 40% in size and 70% in volume, 12 weeks after the end of the treatment. This response was rapid, reproducible and without toxicity.

A controlled study concerning 44 patients with sub-cutaneous radiofibrosis following conservative breast cancer treatment and treated with bovine SOD incorporated in a polyethylene glycol ointment was conducted in 1992. An original histological grading score of the fibrosis by the red picrosirius method on skin punch biopsies taken before and after completion of the treatment showed that the fibrosis grading significantly decreased in 74% of the patients. Immunohistochemistry demonstrated no significant changes in vimentin or TGF-β expression after treatment with SOD, but an increase of the SMC α-actin positive fibroblastic phenotype.

3) Gene expression studies

Sixteen pigs have been gamma irradiated, using a ^{192}Ir source and doses of 2 to 48 Gy were delivered to the flank of the animals. In these conditions, erythema appeared after 32 Gy, and dry desquamation of the epidermis as well as skin and muscular fibrosis developed after 48 Gy. The irradiated samples were removed at 2 h, 15 h and 24 hours after irradiation. Control skin was removed from the non irradiated flank of the same animal. RNA was isolated from these samples, and analyzed by Northern blotting.

Collagen, actin and TGF-β: These genes have been studied because we have previously shown that they play a key role in the formation of radiation-induced scar fibrosis. Their mRNA contents were measured in the samples as a function of the dose and of the time. We found that the alterations in their gene expression occured immediately, as, 6 hours after irradiation, an increased amount of their mRNA was concomitantly observed. Moreover, such an increase was significant only after the high doses which can induce fibrosis.

c-*jun* and c-*fos* transcription factors: We searched whether these factors might be involved in the regulation of the transcription of the collagen, actin and TGF-β genes. For that purpose, their mRNA contents were analyzed in the irradiated samples. We found a significant relation between the c-*jun* factor and the genes involved in the fibrotic process, as its activation was observed for the same doses and at the time than these genes. The activation of the c-*jun* gene was specific for the high doses, as it was never found at doses smaller than 16 Gy. Concerning the c-*fos* factor, a very high induction of this gene was observed in the irradiated samples at high doses (up to 50 times). Consequently, we propose that the trancription factor AP-1, which is composed of the dimer c-jun/c-fos, is involved in this very early response of the skin cells to radiation. Moreover, c-*fos* was induced alone at smaller doses, suggesting a specific role for this factor at low doses.

4) Perpectives

Irradiated skin, subcutaneous and skeletal muscle functions and metabolism will be studied combining gamma scintigraphy, and NMR imaging and spectroscopy, in pigs and rabbits *in vivo* and *ex vivo* after surgical biopsies in irradiated areas will be correlated with objective histological grading and biochemical analysis of proline/hydroxyproline ratio. These parameters will be assessed with and without pharmacological treatments (topical or systemic) and/or decontamination processes.

The mechanisms of SOD action will be assessed in two models. *In vivo*, in the experimental model, SOD treated fibrotic tissues will be surgically removed and compared to untreated fibrotic tissues. *In vitro,* fibroblasts derived from both human and pig fibrotic tissues will cultivated and submitted to SOD treatment for 24 hours. The modifications induced by the treatment will be studied in the following parameters: oxydative metabolism of the cells, activities of synthesis and degradation of the extra-cellular matrix; synthesis of growth factors.

Early gene expressions are currently investigated both in vivo on pig skin and in vitro on cultured human and pig cells. The activities of transcription factors will be more assessed.

A reconstituted skin model was developed with human and porcine keratinocytes and fibroblasts, in order to study changes in the interactions between the different cells, gene expression and protein synthesis after *in vitro* irradiation.The growth factors TNF and EGF expression will be currently studied, as well as the transcription factor p53 and the differentiation markers keratins and involucrin.

5) Publications

Wegrowski J., Lefaix J-L., Lafuma C. Accumulation of glycosaminoglycans in radiation-induced muscular fibrosis. **International Journal of Radiation Biology, 1992, 61, 5, 685-693.**

Martin M., Pinton P., Crechet F., Lefaix J-L. Ionizing radiation regulates expression of c-jun protooncogene and TGFb1 growth factor gene in normal skin. **Journal of Investigative Dermatology, 1992, 98, 5, 820.**

Lefaix J-L. Pathological features of cerebral radiation necrosis. **Part I.** Cerebral radiation necrosis in experimental animals. **Bulletin du Cancer / Radiothérapie, 79, 125-135.**

Lefaix J-L. Pathological features of cerebral radiation necrosis. **Part II.** Cerebral radiation necrosis in man. **Bulletin du Cancer / Radiothérapie, 1992, 79, 251-270.**

Martin M., Lefaix J-L. Fibrose radio-induite: aspects cliniques, cellulaires et moléculaires. Introduction: la fibrose cicatricielle radio-induite. **Radioprotection, 1992, 27, 2, 174-180.**

Lefaix J-L., Daburon F., Fayart G. Dosimetry study of an accidental overexposure to 192 Ir gamma rays. **Health Physics, 1992, 63, 6, 692-694.**

Lefaix J-L., Daburon F., Tricaud Y.. Evolution radiopathologique spontanée et après traitement médical dans deux modèles d'accident d'irradiation localisée. **Bulletin du Cancer / Radiothérapie, 1992, 79, 189-198.**

François-Joubert A.., Leviel J-L., Lefaix J-L., Lebail J-L. In vivo study of rabbit irradiated skeletal muscle by NMR imaging. **British Journal of Radiology, 1992, 65, congress supl., 67.**

Delanian S., Martin M., Lefaix J-L. TGFb1 collagen I and III gene expression in human skin fibrosis induced by therapeutic irradiation. **British Journal of Radiology, 1992, 65, congress supl., 82-83.**

Sabatier L., Martin M., Crechet F., Pinton P., Dutrillaux B. Chromosomal anomalies in radiation-induced fibrosis in the pig. **Mutation Research, 1992, 284, 257-263.**

Martin M., Lefaix J-L., Pinton P., Crechet F., Daburon F. Temporal modulation of TGFb1 and b-actin genes expression in pig skin and muscular fibrosis after ionizing radiation. **Radiation Research, 1993, 134, 63-70.**

Delanian S., Martin M.., Housset M.. La fibrose iatrogénique en cancerologie (I): aspects descriptifs et physiopathologiques. **Bulletin du Cancer , 1993, 80, 192-201.**

Delanian S., Lefaix J-L., Housset M. La fibrose iatrogénique en cancerologie (II): principales étiologies et possibilités thérapeutiques. **Bulletin du Cancer , 1993, 80, 202-212.**

Lefaix J-L., Delanian S., Tricaud Y., Martin M., Hoffschir D., Daburon F., Baillet F. La fibrose cutanéo-musculaire radio-induite (III): efficacité thérapeutique majeure de la superoxyde dismutase Cu/Zn liposomiale. **Bulletin du Cancer , 1993 sous presse.**

Lefaix J-L., Martin M., Tricaud Y., Daburon F. Muscular fibrosis induced after pig skin irradiation with single doses of 192 Ir gamma rays. **British Journal of Radiology, 1993, 66, 537-544.**

Martin M., Pinton P., Crechet F., Lefaix J-L., Daburon F. Preferential induction of c-fos versus c-jun protooncogene during the immediate response of pig skin to gamma rays. **Cancer Research, 1993, 53, 1-4.**

Martin M. Early induction of transcription factors by ionizing radiation: example of the skin radiation response. In: Molecular mechanism in radiation mutagenesis and carcinogenesis, K. H. Chadwick ed., **in press.**

Benyahia B., Magdelenat H. Immunohistochemical characterization of human g-irradiated skin. **Bulletin du Cancer , 1993, 80, 126-134.**

Head of project 2: **Dr. J.W. Hopewell**

II. Objectives of the reporting period

The principle objective of the reporting period 1st September 1992 - 31st August 1993 has been to undertake studies related to "The prevention of early and late radiation-induced sequelae in the skin". The potential beneficial effects of the use of essential fatty acids (EFAs), particularly gamma-linolenic acid (GLA), have been evaluated since they increase the production of PGE_1 which has a number of desirable physiological actions. These include anti-inflammatory, anti-aggregatory and vasodilatory activity. These studies have been undertaken on pig skin, a species whose skin is the most similar to human skin.

In the next reporting period, efforts will be devoted to the detailed evaluation of data to establish dose-effect relationships for non-uniform exposures at low-dose-rates from $^{90}Sr/^{90}Y$ sources for both early moist desquamation and late dermal atrophy. Such data is required for the continued improvement in radiological protection guidelines for the skin. Preliminary studies will also be undertaken to determine the role of increased adhesion molecule expression on endothelial cells in the pathogenesis of both early and late radiation damage to the skin.

III. Progress achieved including publications

To assess the ability of EFAs to ameliorate early and late radiation-induced injury to the skin the effects of two orally administered oils So-1100 [containing linoleic acid (LA) and GLA] and So-5407 [containing eicosapentaenoic acid (EPA) in addition to LA and GLA] were evaluated. Results were compared with animals receiving a 'placebo' oil, So-1129, which contained a similar amount of LA to the other two 'active' oils but no GLA or EPA. The oils were supplied by Scotia Pharmaceuticals Ltd. (Guildford, U.K.).

Groups of animals were allocated to receive the 'active' and 'placebo' oils according to the protocols in Table 1. The oils were given daily, orally both before and after irradiation with single doses of $^{90}Sr/^{90}Y$ ß-rays from 22.5mm diameter plaques at a dose-rate of ~3.0Gy/min, measured at 16µm depth. Skin sites, 16 per flank, were irradiated on each pig.

Following irradiation the severity of the acute skin reaction was assessed at weekly intervals for 10 weeks. Sites were examined for the presence or absence of bright red erythema and/or moist desquamation. Late reactions were evaluated over the period 10-16 weeks after irradiation by assessing the presence or absence of dusky/mauve erythema and/or dermal necrosis. The data for each of these criteria were analysed using probit analysis and ED_{50} (±SE) values, the doses required to cause the effect in 50% of skin fields irradiated, were calculated. These values were used as a means of comparing different treatment schedules.

The ED_{50} values (±SE) for the endpoints of bright red erythema and moist desquamation for pigs receiving various doses of the 'active' oil, So-1100, and the placebo oil, So-1129, are given in Table 2. In the largest series of animals which received 3.0ml of the oils daily, administration of So-1100 as compared with So-1129 for just 4 weeks prior to irradiation had no significant effect on the dose-effect relationship for either the incidence

of bright red erythema or moist desquamation. This lack of effect was reinforced by a comparison with historical data for moist desquamation, where the ED_{50} (±SE) was 27.32 ± 0.52Gy, intermediate between the values of present 26.00 ± 1.87Gy and 27.91 ± 1.15Gy for So-1100 and So-1129, respectively.

Table 1. Experimental design for studies involving the irradiation of pig skin with single doses of $^{90}Sr/^{90}Y$ ß-rays

Experimental Group	Oil	daily dose (ml)	Number of animals
A	So-1100	1.5	4
B	So-1100	3.0	6
C	So-1100	6.0	4
D	So-5407	3.0	2
E	So-1129	3.0	2
F	So-1129	6.0	4
G	So-1100	3.0	2[a]
H	So-1100	3.0	2[b]
I	So-1129	3.0	2[a]
J	So-1129	3.0	2[b]

[a]Oils only given for 4 weeks prior to irradiation
[b]Oils only given for 10 weeks after irradiation

Table 2. Variation in the ED_{50} values (±SE) observed following single doses of $^{90}Sr/^{90}Y$ ß irradiation for the acute skin reaction of bright red erythema and moist desquamation. Either So-1100 or So-1129 were administered daily for various treatment periods. Dose modification factors (DMFs) are quoted (±SE) when the difference between ED_{50} values for the two oils was significant.

Treatment Period (wks)	Oil Dose (ml)	ED_{50} ±SE So-1100	ED_{50} ±SE So-1129	DMF (±SE)
i) Bright red erythema				
-4/+16	1.5	35.9 ± 1.2	-	-
-4	3.0	26.81 ± 1.15	29.68 ± 1.68	NS
-4/+16	3.0	39.23 ± 0.98	31.76 ± 1.39	1.24 ± 0.06
+10	3.0	41.16 ± 3.79	34.44 ± 2.35	1.20 ± 0.14
-4/+16	6.0	35.05 ± 1.12	38.71 ± 1.47	NS
ii) Moist desquamation[a]				
-4/+16	1.5	28.3 ± 0.93	-	-
-4	3.0	26.00 ± 1.87	27.91 ± 1.15	NS
-4/+16	3.0	33.81 ± 0.8	30.04 ± 1.18	1.13 ± 0.05
+10	3.0	31.74 ± 1.08	31.03 ± 1.42	NS
-4/+16	6.0	33.44 ± 0.95	31.22 ± 0.94	NS

[a]Historical control value, no oils, ED_{50} (±SE) 27.32 ± 0.52Gy

Administration of 3.0ml of oils, daily, for 4 weeks prior to irradiation and over the time course of the acute skin reaction, produced a significant modulation ($p<0.05$) of the radiation response in animals receiving So-1100 as compared with So-1129. Dose modification factors (DMFs) of between 1.13 and 1.24 were obtained. There was also a suggestion of an effect produced by the 'placebo' oil, So-1129, since the ED_{50} values for both bright red erythema and moist desquamation, when this oil was given over the time course of the reaction, were higher than when this oil was just given prior to irradiation. For moist desquamation, where a comparison with historical control data from pigs receiving no oils is possible, a significant effect of the 'placebo' oil is suggested with DMFs of 1.1 ± 0.04 and 1.14 ± 0.04 when compared with the -4/+16 week and +10 week So-1129 groups, respectively. Further evidence for a 'placebo' effect were supported by the results obtained when pigs were given daily doses of 6.0ml of each oil; the greater modulation produced by So-1100 relative to So-1129 was not seen. Administration of a lower dose of 1.5ml of So-1100 modified the expression of the inflammatory bright red erythema response but not the severity of moist desquamation.

For the later dermal reactions of dusky/mauve type erythema and ischaemic dermal necrosis, daily doses of 3.0ml of So-1100 also produced a significant modification in the radiation response when results were compared with those seen in the 'placebo' group (Table 3). However, the oil had to be given over the time course of the radiation response to be effective. No significant effect was seen when So-1100 (3ml/day) was only given prior to irradiation. There was evidence, indicative of a 'placebo' effect, when So-1129 was given both before and after irradiation at a dose of 6.0ml/day. The ED_{50} values were significantly higher than when the same oil was given at 3.0ml/day and for historical data for pigs receiving no oil ($p<<0.001$). At this higher dose of oil there was no significant additional gain obtained from the use of So-1100; DMFs were not significantly different from 1.0 (Table 3). For these later dermal vascular reactions, 1.5ml/day of So-1100 produced a significant modulation of the expression of radiation damage when compared with the combined data for pigs receiving 3.0ml/day of So-1129. The DMFs of 1.26 ± 0.09 and 1.16 ± 0.08 were not significantly different from those obtained using 3.0ml/day of So-1100 both before and after irradiation (Table 3).

Studies involving the oil So-5407 were only carried out using 3.0ml/day, given both before and after irradiation. The results of these investigations are given in Table 4. For the early endpoints there appears to be a modification in the response seen when compared with a 'placebo' group, however, the difference in ED_{50} values suggesting DMFs of 1.14 ± 0.08 and 1.15 ± 0.08, respectively, only approached statistical significance ($p<0.1>0.05$).

Significant modification of the later dermal reactions was seen after the administration of So-5407 over the time course of the reaction as compared with So-1129. These changes were consistent with DMFs of 1.32 ± 0.13 and 1.2 ± 0.12, for dusky/mauve erythema and ischaemic dermal necrosis, respectively.

The DMFs noted following irradiation with single doses of $^{90}Sr/^{90}Y$ ß-rays using the oil So-5407 were in the same range as those found using So-1100. Neither agent was found to act as a classical radioprotector but had to be given over the time scale of the expression of radiation damage and hence could be used in the prophylactic treatment of radiation accident victims. They have been administered to humans over periods up to 1 year without significant adverse side effects.

Table 3. Variation in the ED_{50} values (±SE) for the later dermal reactions of dusky/mauve erythema (DE) and ischaemic dermal necrosis (N) after the administration of either So–1100 or So–1129 for various time periods following irradiation with single doses of $^{90}Sr/^{90}Y$ ß rays. DMFs are quoted (±SE) when the difference between the ED_{50} values for one to two oils was significant.

Treatment Period (wks)	Oil Dose (ml)	ED_{50} ±SE So–1100	ED_{50} ±SE So–1129	DMF (±SE)
i) Dusky/mauve erythema (DE)[b]				
–4/+16	1.5	32.96 ± 1.61	–	1.26 ± 0.09[a]
–4	3.0	26.5 ± 1.3	27.5 ± 1.1	NS
–4/+16	3.0	37.53 ± 1.89	24.84 ± 1.53	1.35 ± 0.11
–4/+16	6.0	37.97 ± 1.82	38.44 ± 2.08	NS
ii) Dermal necrosis (N)[c]				
–4/+16	1.5	40.95 ± 2.12	–	1.16 ± 0.08[a]
–4	3.0	34.8 ± 1.4	35.0 ± 1.5	NS
–4/+16	3.0	40.64 ± 1.31	35.7 ± 1.55	1.14 ± 0.06
–4/+16	6.0	49.56 ± 3.01	51.93 ± 3.87	NS

[a]DMFs determined by a comparison with the combined data for 3.0ml of So–1129
[b]Historical control value, no oil, 25.56 ± 5.66Gy
[c]Historical control value, no oil, 35.34 ± 4.02Gy

Table 4. Variation in ED_{50} values (±SE) for the acute skin reactions of bright red erythema (C) and moist desquamation (MD) and the later dermal reactions of dusky/mauve erythema (DE) and necrosis (N) after single doses (SD) of $^{90}Sr/^{90}Y$ ß irradiation. DMFs are quoted for (±SE) for the comparison between So–5407 and So–1129.

Reaction type	ED_{50} ±SE So–5407	ED_{50} ±SE So–1129	DMF ±SE
C	36.23 ± 1.97	31.76 ± 1.39	1.14 ± 0.08
MD	34.6 ± 1.85	30.04 ± 1.18[a]	1.15 ± 0.08
DE	32.88 ± 2.54	24.84 ± 1.53[b]	1.32 ± 0.13
N	42.74 ± 3.9	35.7 ± 1.55[c]	1.20 ± 0.12

[a]Historical control value, no oils 27.32 ± 0.52Gy
[b]Historical control value, no oil, 25.56 ± 5.66Gy
[c]Historical control value, no oil, 35.34 ± 4.02Gy

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1992:

Rezvani, M., Nissan, M., Hopewell, J.W., van den Aardweg, G.J.M.J., Robbins, M.E.C. and Whitehouse, E.M. Prevention of X-ray-induced late dermal necrosis in the pig treated with monochromatic light. *Lasers Surg. Med.* **12**, 288-293, 1992.

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Rezvani, M., Robbins, M.E.C., Hopewell, J.W. and Whitehouse, E.M. Modification of late dermal necrosis in the pig by treatment with multi-wavelength light. *Brit. J. Radiol.* **66**, 145-149, 1993.

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Hopewell, J.W., Robbins, M.E.C., van den Aardweg, G.J.M.J., Morris, G.M., Ross, G.A., Whitehouse, E., Horrobin, D.F. and Scott, C.A. The modulation of radiation-induced damage to pig skin by essential fatty acids. *Brit. J. Cancer,* **68**, 1-5, 1993.

CEC Report September 1993 Head of Project 3: Dr. Coggle.

II. Objectives of the reporting period

To complete the study of the synergy between localised Sr-90 beta irradiation of mouse skin and whole body X-irradiation.

To confirm that CBA mice are markedly less skin cancer prone than CD1 mice.

To develop and apply molecular techniques to investigate the roles of TGF-β, TNFα, *c-fos* and *c-jun* in the pathogenesis of radiogenic skin cancer induction in our mouse model.

III. Progress achieved including publications.

The details of the study, in the light of Chernobyl and Goiania accidents, into the effects of whole body (WB) X -irradiation on the severity and time course of an acute skin reaction induced by localised Sr-90 beta exposure are given in the reference, Randall and Coggle (1992). The paper suggested the lack of synergy was due to a mismatch between the timing of the immunosuppression (2-10 days) and of the moist desquamation (10-25 days). To test this idea groups of SAS/4 mice were given 50 Gy of Sr-90 betas over a 2×4 cm area of flank skin followed 7 days later by 4, 6, or 8 Gy WB doses of X-ray. This caused the nadir of the immune the suppression at 10-20 days, to coincide with the timing of acute skin reaction. This had no effect on time of onset of the skin reactions, but the plateau's and the final resolution of the lesions were significantly prolonged by the WB doses. Thus the timing of the peak reactions in the 0 Gy group (betas only); the 4, 6, and 8 Gy whole body groups was 15-25; 15-28; 15-35 and 15-40 days respectively. It seems that the immune system plays a significant role in this synergy.

Short and long term investigations of gene expression

Short and long term investigations of gene expression are in progress in two different strains of mouse; an outbred albino strain, the CD1 mouse and an inbred pigmented strain, the CBA/ca mouse. The two strains have previously been shown to have differing tumour proneness after irradiation. The more cancer prone CD1 mice also showing a higher proportion of dermally derived tumours.

There is an increasing awareness of the role of cytokines in the regulation of cell function, including growth and tissue homeostasis and consequently their role in perturbation. For this reason it was decided to study the expression of a series cytokines in mouse skin after radiation damage. Transforming Growth Factor β (TGFβ), Tumour Necrosis Factor α (TNFα), Interleukin 1α (Il1α) and Interleukin 6 (Il6) have been chosen for study initially but our current technique for following gene expression will allow rapid analysis of a wide range of cytokines as the need for their investigation becomes apparent. The expression of proto-oncogenes *c-fos* and *c-jun* will also be investigated due to their important role in the response to DNA damage and their potential action as transcription factors which may induce some of the cytokines of interest.

A range of times post irradiation have been selected for study. These encompass responses taking place within the first few hours (1-6), days (1-2) and weeks (1-2), and also the first six months. Mice were exposed to a 50 Gy dose of Sr-90 over an area 11mm in diameter. Groups of five mice were then sacrificed at the appropriate time after irradiation. 50-100 mice from both strains were irradiated in the same way and left to produce tumours.

Sample collection for the pigmented CBA/ca mice, involved in the long term study is complete. Analysis of the data shows a 63.8% +/-7(S.E) value for percentage cumulative tumour incidence. Tumours began to appear 50 weeks after irradiation with the maximum occurrence of new tumours being between 70 and 80 weeks. Those tumours that have been produced are awaiting histological analysis. Work is in progress to assess the gene expression in these tumours and this will be analysed in conjunction with the histopathology. Gene expression in the non-tumour bearing fibrotic scars of 70 week old mice onwards is also being analysed and compared to unirradiated skin of the same age. Preliminary results show very high levels of TGFβ expression and significant levels of TNFα in the tumours compared to both the remaining scar tissue and the unirradiated control skin of the same age. The tumours produced by CBA/ca mice have also been used to produce lines of transplanted tumours over five generations to date. This work is ongoing and it is hoped to investigate gene expression in tumours that are becoming progressively more aggressive at each generation. TGFβ again appears to be highly expressed and the initial results suggest increasing expression of TNFα

During the early stages of the research and for the majority of the early time points attempts were made to analyse gene expression by northern blotting and hybridisation techniques. This particular method was, for mouse skin at least, found to be very unreliable. Recent advances in molecular biology techniques have made it possible to analyse RNA expression using a reverse transcription polymerase chain reaction. In recent months this has been the method of choice when studying the expression of cytokines in mouse skin. This method can be used both qualitatively for phenotypic analysis of a range of cytokines and quantitatively using an internal standard.

RNA expression profiles have been completed in both strains of mouse at 2, 4, 8, and 12 weeks after irradiation for Il1α, Il6, TNFα and TGFβ. A striking interstrain difference has been found in the expression of TNFα. Expression is apparently constitutive in the CD1 mice over this period. However,in the CBA/ca mice transcripts are only detectable 14 days after irradiation (for the times studied). Constitutive expression is apparent for the other aforementioned genes in both strains. Preliminary results suggest that levels of all are elevated above that of the controls by 14 days and remain so until one month after irradiation. After this time the levels begin to fall although they remain above those of the controls. Quantitative analysis is in progress to assess relative changes in gene expression both over time and between strains. Further work is also required to elucidate at what stage levels of gene expression begin to increase.

Publication:

Randall, K and Coggle, J., The Effect of Whole Body γ-irradiation on localised β-irradiation induced skin reactions in mice. International J. Radiation Research 62 729-733 (1992)

IV. Objectives of the next reporting period.

1. Continue to assess gene expression in both primary skin tumours and the transplanted F_1 to F_x cell lines derived from them.

2. To develop and apply the quantitative PCR methods to 'normal' mouse skin at times after radiation ranging from a few hours to 1-2 years.

3. To initiate immunohistochemical studies of the protein products of some of the growth factors using *in situ* hybridisation.

Head of project 4: Dr. Di Carlo

II. Objectives for the reporting period

The first step is the clinical classification of the radiation workers. These are subdivided in two subgroups: those with clinical evidence of radiodamage, represented by scleroatrophy of the fingers, marked onicodistrophy, and those working in radiation field from more than 20 years, with minimal or not clinical evidence of radiolesions, such as scleroatrophy and sclerodistrophy. If necessary both histological and ultrastructural examination are performed. As control we select normal subjects, no smokers, no hypertensive or diabetic, aging 20-60 years.
The aim is to establish, by means of thermographic technique, possible differences in terms of times of thermal recovery (TRT) after thermostimulation (+5°Cx20") between healthy subjects and apparently normal radiation workers.

III. Progress achieved including publications

We have evaluated 20 normal subjects: a very little difference (less than 20") has been observed as regard sex, side and single fingers. The range of TRT varied from 2'and 6'(+-40") in 16 subjects, 30"-1'in 3 subjects, and over 7'in 1 subject. In one case of chronic professional radiodermitis with severe clinical alterations, the affected fingers showed a significant difference with the other fingers, with a very prolonged TRT (over 10'). Histological and ultrastructural examination of these fingers showed numerous vessels with a fibrous thickening of the walls, so that the lumen was nearby occuded. In 20 radiation workers without important skin modifications, times between 2'and 6'(+-40") have been observed in 13 subjects, over 6'40" in 5 subjects, and between 30" and 1'20" in 2 subjects; only in one patient we found a significant difference of TRT of the fingers in the same subject.
Ippolito F.,Strambi E.,Di Carlo A.,Palma G. Thermographic examination with thermostimulation in radiation workers. A clinico-epidemiological study (in press)

Progress Report

Contract: FI3P-CT930076 Sector: B24

Title: Thyroid and its proximate tissues radiation dosimetry; stochastic and deterministic biological effects in humans and model systems.

1) Lamy — Univ. Bruxelles (ULB)
2) Malone — Hosp. Federated Dublin Voluntaries
3) Smyth — Univ. Dublin - College
4) Williams — Nat. School of Medicine

I. Summary of Project Global Objectives and Achievements

This thyroid project as a whole has the objective of providing a more reliable scientific background against which public health officials radiation protection organisations and governments may evaluate risks from thyroid irradiation, establish dose limits, intervention levels and monitoring techniques appropriate to normal time and/or in the event of an accident. Within this general framework it is expected to :

a) advance foetal and neonatal thyroid dosimetry, and the dosimetry for sensitive target organs/tissues (e.g. brain), liable to be irradiated by radionuclides contained in the foetal thyroid. **Foetal thyroid dosimetry has been established by experiments on phantoms and the MIRD scheme validated.**

b) render more reliable the iodine kinetics models which underly foetal and neonatal dosimetry. **Iodine metabolism studies have revealed an important renal iodide loss in pregnancy.**

c) use newly created animal models and develop new animal models of transgenic mice for the study of the carcinogenetic process in thyroid in vivo and the role of environmental factors (eg. iodine supply, level of stimulation, etc). **New animal models of transgenic mice with undifferentiated carcinoma (SV40 LT transgenes), partially differentiated adenomas (HPV16 E7 transgenes), and hyperfunctioning adenomas (adenosine A2 receptors) have been established.**

d) establish issue culture models for radiation induced thyroid diseases (including neoplasia). **Culture models of dog and human thyroid cells have established the cAMP cascade as mitogenic and differentiating. In agreement with this concept new models of human cell line obtained by transfection and expressing Adenosine A2 receptors and HPV16 E6 and E7 genes have been developed. It has been shown that a mutation conferring constitutive activation to the TSH receptor account for 40% of thyroid hyperfunctioning adenomas.**

Head of project 1: Prof. Lamy

II. Objectives for the reporting period

1) Development of a human thyroid cell line : attempts using new oncogenes and vectors.

2) Development of transgenic mice as models for human thyroid tumorigenesis: development of a line based on human papillovirus 16 E6, and further development of the model of autonomous adenoma based on adenosine A2 receptor.

3) Definition of the mechanism of proliferation control in the human thyroid tumor cell : search for genetic lesions leading to autonomous hyperfunctioning adenoma.

III. Progress achieved including publications

1) Development of human thyroid cell line

In one of the numerous attempts to create a new human thyroid cell line, we have succeeded in creating such a line using a pML2 vector with immortalizing HPV16 E6 and E7 genes under the control of the powerful CTR promoter of Mo Mu. This line restrained some differentiation characteristics of thyroid cells : thyroglobulin synthesis and secretion, cyclic AMP response to TSH, and thyroglobulin secretion in response to TSH. It did not retain the TSH control of proliferation. Unfortunately after passage 22 the cell strain lost its differentiation characteristics. We have started a new program of transfection using recombinant HPV16 DNA, and pML2 vector with : HPV16 E6 gene under the control of a thyroglobulin promoter, HPV16 E7 gene under the control of a thyroglobulin promoter, E6 and E7 under the control of a SV40 promoter, or of a thyroglobulin promoter, constitutive adenosine A2 receptor under the control of a SV40 or a thyroglobulin promoter. Cells have been cultured alternatively in the presence of thyrotropin and forskolin so as to eliminate fibroblasts and favor cells with a mitogenic TSH-cyclic AMP pathway. In such preparations many cells stop proliferating and die. In three case continuously growing cells have been obtained : i.e. in cells transfected by the cDNA of the adenosine A2 receptor and in the cells transfected with HPV16. These cells are now being characterized.

2) Development of new animal models of human thyroid tumors : transgenic mice.

Two animal models of human thyroid tumors have been created in transgenic mice. In one, the SV40LT cDNA, under the control of a thyroglobulin promoter induces specifically in the thyroid an undifferentiated non functioning rapidly growing tumor involving the whole thyroid. These whole gland adenomas later degenerate in undifferentiated metastasizing tumors. In the other model, the adenosine A2 receptor under the control of a thyroglobulin promoter induces specifically in the thyroid, an hyperfunctioning adenoma involving the whole gland. The study of these models has been pursued. In particular it has been shown that the adenosine A2 receptor is constitutively active for the adenylate cyclase pathway but not for the PiP2 phospholipase C cascade. The hyperfunctioning adenomas of the adenosine A2 mice therefore entirely result from the constitutive activation of the cyclic AMP cascade. During the last year a new model of transgenic mice has been created using the HPV16-E6 immortalizing gene under the control of a thyroglobulin promoter.

These animals develop a progressively growing goiter. The function of the thyroid, as evaluated from serum thyroid hormone measurements is normal. Taking into account the greatly increased number of cells this demonstrates a lower functional activity per cell. However whether this is due to some functional dysfunction or to a lower level of TSH stimulation is not known. These mice do not develop malignant tumors. Transgenic mice expressing both E6 and A2 receptor have been generated. These mice present a combined phenotype with addition of the effects of each protooncogene : the growth as well as the hormone secretion of the thyroid is greatly increased in double transgenics. This shows that the E7 pathway is not dedifferentiating (as the FGF, HGF growth pathways). New E6 transgenic mice have just been generated but not characterized.

3) Definition of the mechanism of proliferation control in the human tumor cell.

Our group has demonstrated the mitogenic, dedifferentiating and function activating role of the TSH cAMP cascade in the thyroid. From this mostly in vitro work, we predicted that chronic stimulation of this cascade should lead to hyperfunctioning adenoma. This hypothesis has been tested in human thyroid "hot" nodules, i.e. hyperfunctioning adenomas. In the investigation of the first levels of the cascade, the TSH receptor, we demonstrated somatic mutations in 4/11 cases. In these cases, the TSH receptor was mutated in the adenomatous but not in the normal tissue. This mutation has been demonstrated by transfection of the corresponding cDNA in COS 7 cells to confer constitutive activation vs adenylate cyclase to the receptor. We are now investigating other types of tumors.

PUBLICATIONS

PARMA, J., DUPREZ, L., VAN SANDE, J., COCHAUX, P., GERVY, C., MOCKEL, J., DUMONT, J.E., VASSART, G.
Somatic mutations in the thyrotropin receptor gene are responsible for hyperfunctioning thyroid adenomas.
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Thyroid expression of an A2 adenosine receptor transgene induces thyroid hyperplasia and hyperthyroidism.
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WALLACE H., LEDENT, C., VASSART, G., BISHOP, J.O.,AL-SHAWI, R.
Specific ablation of thyroid follicle cells in adult transgenic mice.
Endocrinology 129 (6) 3217-3226, 1991.

CEC Radiation Protection Research Action
Project: FI3P - CT930076 (Thyroid)

First Report From: Prof. J.F. Malone

The overall project objectives as outlined in the initial proposal are as follows:

* Advance foetal and neonatal thyroid dosimetry, and the dosimetry for sensitive target organs / tissues (eg. brain), liable to be irradiated by radionuclides contained in the foetal thyroid, or maternal organs.

* Derive a more scientific reliable and easier to measure unit than radiation dose against which risks can be assessed in the thyroid.

* Using newly available data on thyroid mass and iodine kinetics, provide country by country (or region) maps for the probable distribution of thyroid radiation dose throughout Europe.

In the first reporting period it was hoped to reach the following stages in the sections listed above:

* Reestablish the work on the thyroid dosimetry.

* Construct phantoms simulating the relative positions of the foetal thyroid and other relevant foetal organs to allow dosimetry computations to be checked.

* Initiate work on the calculation of detriment arising from an injection of I-131.

* Investigate the current unit and its inherent weakness and make progress on the development of a new unit.

* Initiate the design of a database on thyroid mass and iodine kinetics throughout Europe for use with the development of dosimetry maps.

Progress Report for the First Reporting Period:

* The work on thyroid dosimetry has been reestablished taking into account the relevant data on thyroid mass and iodine kinetics as developed by P. Smyth within this project.

* Fetal thyroid dosimetry has been extensively modelled. One of the major weaknesses in the present fetal dosimetry is the fact that the estimates of brain dose from fetal thyroid irradiation are based on calculation only and have not been subject to direct measurements. To this end a simple practical volumetric phantom has been constructed and used to estimate maximum, minimum and average 'S' factor profiles of fetal brains from I-131 in the fetal thyroid at four stages of gestation, (12,16,24 and 36 weeks). The results obtained agree with those estimated using the MIRD scheme and traditional methods.

At present lack of tissue equivalence in the phantom would have contributed to errors in each 'S' factor measurement. Also, the phantom used in this experiment does not simulate the effect of fetal bone structures such as the fetal mandible or fetal skull on dose. These structures will increase scatter near the tissue bone boundaries. However, having regard to the errors described, it can be assumed that the MIRD approximation method and the traditional methods are reasonable methods of estimating dose for certain fetal organs.

* Based on the reports of ICRP and UNSCEAR, progress has been made on the calculation of the detriment arising from ingestion of I-131. The results show that the risk of mental retardation threshold is exceeded only when activities within the medical therapeutic range have been ingested. In terms of fetal detriment, the structures at the base of the brain are likely to be more at risk than the developing forebrain because of their proximity to the thyroid and the later gestational development of these structures.

* It is felt that both the organ and the integral absorbed dose may not be appropriate concepts for use in risk estimates as they do not take account of the number of cells being irradiated or microdosimetric considerations. An alternative concept for use with risk estimates has been derived. The proposed concept for use in risk estimation is consistent with the assumption that the risk of transformation is directly related to the number of cells at risk, whereas other existing methods of risk estimation that have been examined are not consistent with this relationship.

The probability of a cell undergoing initiation in a gland will increase with energy deposited in each cell. It is not unreasonable to assume that initiation is a stochastic process with no threshold. If this is the case the number of cells undergoing initiation will increase with the number of cells in which energy is deposited. This hypothesis contradicts the conventional method of risk estimation which assumes dose and risk will increase with energy deposited per cell but takes no account of the increased risk from depositing energy in a greater number of cells.

The use of a more versatile concept such as energy imparted per cell summed over the total number of cells and adjusted for any edge effects such as beta escape from the gland will provide a complete picture of deposition of

biologically effective energy in the gland. The energy imparted per cell is the product of the absorbed dose in the cell and the mass of the cell. Work is in progress to establish if an effective transfer from this level to the world of macroscopic units can be credibly established.

* The database necessary for the compilation and analysis of regional data has been developed with the collection of relevant data initiated.

Work schedule for the second reporting period:

* Incorporate the results on thyroid mass / volume and iodine kinetics from P. Smyth's work and account for same in dosimetry work.

* Continue phantom measurements with further refinements in phantom structure and measurement techniques.

* Modify existing detriment determination to incorporate the results from studies on iodine kinetics carried out by P. Smyth.

* Initiate studies and surveys of thyroid natural history to examine possibility of alternative unit.

* Input the accumulated data on iodine kinetics, thyroid mass / volume, regional iodine distribution and detriment calculations into the developed database. Use database to initiate development of regional map.

Publications:

Malone JF., Unger J., Delange F., Lagasse R., Dumount JE., 1991. Thyroid Consequences of Chernobyl in the countries of the European Community, J. Endocrinol. Invest., 14, 701-717.

Gilligan P. and Malone JF., 1991. Radiology of the Thyroid: Estimates of Consequences of Radioiodine Fallout. In proceedings of EORTC Study Group, Dublin, 1992. (Berlin, Henning).

Malone JF., 1992. Consequences of Iodine Fallout: Dosimetric and Radiobiological Considerations. In "Iodine Deficiency in Europe, a Continuing Concern", Ed. F. Delange et al. (New York, Planum Press).

Head of project 3: Dr. Smyth

II. Objectives for the reporting period

1. To initiate a study examining prospective changes in maternal renal handling of ingested iodine during the three trimesters of pregnancy and postpartum.

2. To investigate the iodine content of breast milk from nursing mothers and to compare findings with other European studies.

3. To establish the relationship between maternal and neonatal urinary iodine excretion in breast feeding and non-nursing mothers.

4. To establish an experimental model whereby the effects of external irradiation on intercellular signalling systems in organ and cell cultures of animal and human thyroid can be determined.

III. Progress achieved including publications

1. Retrospective Study:-

An early report had shown that in contrast to findings in other European countries where iodine intake is low, urinary iodine excretion in the study in the study population increased during the three trimesters of pregnancy. Because this finding was at variance with other reports, the study was repeated in two parts (a) retrospective: Random urine samples were obtained from groups of patients attending a maternity hospital during the first, second and third trimesters of pregnancy (T1, T2 and T3) and six weeks postpartum (PP6). Results were expressed as micrograms of I excreted / gram creatinine are shown in Table 1. As iodine excretion in Northern European countries demonstrates a seasonal variation, results were compared to a controlled non-pregnant female population (N=1063) sampled at different seasons over a two year period.

Group	N	Mean±SE µg/g Creatinine (Median)
Control	1063	100±2.2 (82)
T1	53	186±13.8 (164)
T2	113	156±7.6 (143)
T3	92	159±9.2 (139)
Delivery	62	90±6.8 (77)
PP 6	87	100±6.8 (82)

Table I: Mean values±SE for urinary iodine excretion expressed as µg/g creatinine measured in random urine samples during pregnancy and postpartum.
T= Trimester 1,2,3. PP= Weeks postpartum.

The results confirm the findings in the previous study in that urinary iodine excretion was increased during the three trimesters of pregnancy and declined to control levels at delivery and remained at this level postpartum.

2. Prospective study:

A group of 38 individual pregnant patients from within the retrospective group were available for follow up sequential studies during the three trimesters of pregnancy.

Group	T1	T2	T3
µg/g Creatinine	186±17.5	145±11.2	160±14.4
Median	163	141	135

Table 2: Mean values±SE for urinary iodine excretion expressed as µg/g creatinine in 38 individuals assessed sequentially during the three trimesters of pregnancy.
T= Trimester 1,2,3.

As shown in Table II, iodine excretion in this group paralleled that observed in the retrospective group offering further confirmation that the increase in urinary iodine excretion observed during pregnancy was a real effect which reflected increased renal iodine loss during gestation.

3. Maternal Neonatal Iodine Status::

To determine the relationship between iodine handling in the mother and neonate, a study was commenced whereby urine specimens were obtained from both mother and neonate at three days following delivery. In addition, a specimen of breast milk was obtained from nursing mothers. The objectives of this study are

(a) to determine the relationship between iodine handling in mother and neonate in both nursing and non-nursing mothers.

(b) To examine the role of the breast in concentrating iodine during a period of relative iodine deficiency.

Head of Project 4: Profesor Sir Dillwyn Williams

II. Objectives for the reporting period

To develop techniques for the study of the role of certain oncogene and growth factors in the pathobiology of thyroid cancers.

(a) in situ hybridisation
(b) immunocytochemistry

To begin the application of these techniques to human thyroid cancers and to lesions developed in transgenic mice bearing the constitutively activated A2 receptor.

III. Progress achieved including publications

Techniques have been developed for a sensitive non radioactive in situ hybridisation method applicable to paraffin and frozen sections. This approach, modified from previously published methods has proved reliable and sensitive, and has been published[1]. Probes successfully used include IGF1, EGF, ret, and as markers of tumour differentiation, calcitonin, thyroglobulin and calcitonin. This work has been presented at meetings,[2, 3, 4, 5] and in press,[6], submitted[7, 8] or is in preparation. Immunocytochemical techniques are in use again after transfer of the laboratory to Cambridge, and have been used in combination with ISH in most of the publications referred to. The antibodies in use include IGF, p53, EGF; ret is in process of development. Application of these methods to human and experimental tissue is in progress.

1: Thomas GA, Davies HG, Williams ED (1993) Demonstration of mRNA using digoxigenin labelled oligoprobes for in situ hybridisation in formamide free conditions. J Clin Pathol 46: 171-174
2: Thomas GA, Davies HG, Williams ED (1992) Demonstration of mRNA for EGF and calcitonin using digoxigenin labelled cDNA oligoprobes in a formamide free conditions. 19th Annual meeting of the International Academy of Pathology.
3: Thomas GA, Davies HG, Williams ED (1992) Expression of IGF1 in the thyroid. 165th meeting of the Pathological Society
4: Thomas GA, Neonakis E, Davies HG, Wheeler M, Williams ED (1993) Comparison of somatostatin and calcitonin mRNA and peptide in rat and human thyroid. 21st Annual Meeting of the European Thyroid Association 1993
5: Williams ED, Davies HG, Thomas ND, Thomas GA (1992) Calcitonin transfer from C to follicular cell in the thyroid. An immunocytochemical and in situ hybridisation study. 21st Annual Meeting of the European Thyroid Association 1993

Progress Report

Contract: FI3P-CT920015 Sector: B31

Title: Effects of protracted exposures to low doses of radiations during the prenatal development of the central nervous system.

1)	Reyners	CEN/SCK Mol
2)	Coffigny	CEA - Bruyères-le-Châtel
3)	Ferrer	Hosp. Principes de España

I. Summary of Project Global Objectives and Achievements

The study of the protracted exposures: "A **continuous** (4 day long) exposure of the fetal rat brain to **a low dose** of radiation can be at the origin of a **microcephaly** nearly **as important** as the one caused by the same dose given **acutely** (in a few seconds on day 15 of the pregnancy)." This finding was made under our precedent EEC contract. It was also noticed then that an **acute** irradiation with **neutrons** of 600 keV was 3.5 times more efficient than the X- or the gamma rays; in addition, the "threshold" dose for the neutron microcephaly was very low: 1 cGy (35 mSv; Wistar-cen inbred rats). Because of the neutron efficiency, it was *speculated* that a **protracted** exposure to these particles could also elicit an abnormal brain development *possibly down to doses below 5 cGy*. **To test this hypothesis,** 37 pregnant Sprague-Dawley rats (OFA, Iffa Credo) were irradiated for 4 days, from day 12 to 16 post conception (d12-16pc) and from d16-20pc with Cf-252 neutrons at Arcueil (F). The rats born after these treatments were distributed among the 3 participating laboratories and processed according to their specific assessments.

Mol (CEN-SCK, Hubert REYNERS, coordinator) is responsible for the search on the suspected brain atrophy and the associated anatomical changes, particularly focusing on the development of the gyrus cinguli in the corpus callosum (the main white matter component of the brain). Other specific quantitative assessments involved an analysis of the subsurface cisterns (Electron microscopy) and of the hippocampal synapses (immunocytochemistry.).

Fontenay aux Roses (CEA-FAR, Hervé COFFIGNY, contractant no. 2) is in charge with the implementation and the dosimetry of the neutron exposures as well as with the breeding and fixation of the animals. About 340 rats (including the pregnant dams) were involved. Further on, Coffigny carried out biochemical and survival assessments (in cell cultures of the striatum and of the mesencephalon) on the irradiated neurons and glia (now extended to the perinatal hippocampus) .

Barcelona (Hospital Princeps d'Espanya, Isidre FERRER, contractant no. 3) evaluates the fate of selected classes of neurons in the irradiated young brain. The present report deals with the data of satellite experiments which complement the main neutron project. A comparison is established between the radioinduced cell death and the natural apoptosis of the perinatal period. Ferrer focuses particularly his observations on two brain areas (hippocampus and cerebellum) where cellular growth does not stop after birth and where, according to his views, some restoration could take place.

Head of the project 1: H. Reyners

II. Objectives for the reporting period:

1. LAST PERIOD: A large scale experiment focusing on the brain effects of a protracted exposure (**4 days**) to neutrons during the cerebrogenesis was carried out on 37 Sprague Dawley pregnant rats using 2 series of irradiation carried out at Arcueil (F) in 1992. More than 330 rat pups were born with cumulated doses ranging from 0 to 28 cGy; the minimal dose level was 4 cGy (dose rate: **1 cGy/day**). The female progeny (150) was kept at the CEA-FAR until 3 month old and then processed for further evaluation at Mol. **A range of brain parameters** (detailed below) were assessed. A particular emphasis was actually set on the analysis of the development and of the causes of the **white matter atrophy,** a characteristic of prenatal irradiations also recently encountered after **protracted** low dose exposures.

2. NEXT PERIOD: Mol will perform **a comparative study** of **protracted and acute** neutron irradiation using the same 1 MeV neutrons for the 2 kinds of exposure. The neutrons will be produced at the Van de Graaff accelerator (Dr A. Crametz) of the EEC CBNM in Geel (B). The main focus will be on the mechanisms of the brain damage, in particular the changes in the **thin structure of the myelin** sheaths in the cingulum. The **distribution of the synaptic contacts** in the hippocampus or in the cerebral cortex will also be on focus. These areas are also considered in the behavioral assessments made at the NRPB (UK), a newcomer to the current project. Longer term (3 years) studies with larger doses of 50 to 200 cGy of X rays will be initiated.

III. Progress achieved:

Assessing Microcephaly: A general but curious observation can be done about the effects of the low doses of irradiation (i.e.: <500 mSv) on the developing brain: it is the difficulty of a number of sophisticated, even quantitative, methods to tackle the tissular changes associated with the **brain atrophy** which are revealed by the brain weight measurements down to very low dose levels (35 mSv). The low sensitivity of the other experimental assays is a major drawback as far as the mechanisms of the deleterious effects of the very low doses cannot be fully apprehended or analyzed at the current levels able to induce a brain atrophy. The use of more sophisticated approaches remains thus restricted to higher dose ranges (often as high as 500 and up to 2000 mSv as in Ferrer's present report) at the possible risk of dealing with **slightly different** radiobiological syndromes.

Results: The present study of the effects of the Cf-252 neutrons does not modify the above statements since it also points out to the efficiency of the brain weight measurements as the **best *current* sensor of a general brain atrophy**. The latter parameter is even superior to the Cingulum volume assessment with image analysis by at least one order of magnitude; however, it will be seen that the more specific methodologies reported below (including also the immunocytochemistry of synaptophysin and the electron microscopy of subsurface cisterns) open the way for an interpretation of the **mechanisms** at work.

a) Brain atrophy (this task is 100% completed by now):

1.- *d12-16pc exposures*: As shown in fig. 1a, the brain weights of 103 female rats (fixed for electron microscopic observations at Mol) were found significantly decreased after the continuous neutron exposure (1-way ANOVA analysis). The effect was **significant down to the dose-rate of 1** cGy/**day** (total dose: 4 cGy from d12-16pc) thanks to the large number of rats present in this group (35 animals treated with 1 cGy versus 28 sham treated). The data follow a linear dose-effect relationship although the effects in the higher dose group of 28 cGy appear somewhat lower than expected (but there were only 8 rats available at this "high level"). From these data, a relatively low **RBE of 3.7** was calculated for the brain weight endpoint (respective to a protracted d12-16pc gamma ray exposure).

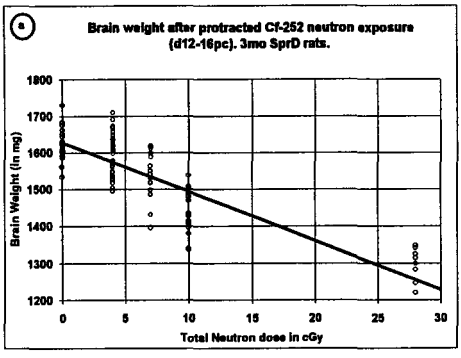
2.- *d16-d20pc exposure: 34* rats were irradiated with total doses of 4 cGy and 10 cGy from d16-20pc. Brain weights were not modified, even after 10 cGy.

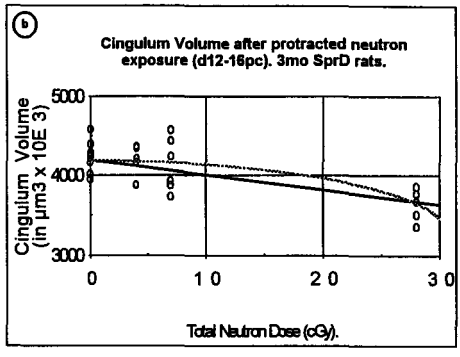
b) White matter atrophy in the cingulum (task 65% completed):

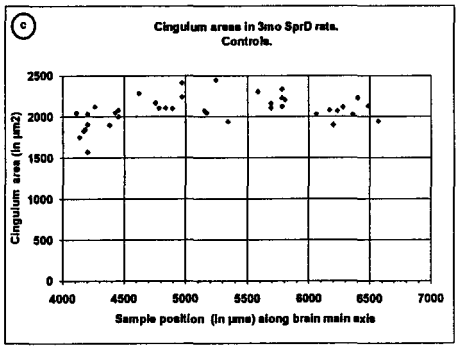
1.- Image analysis results (figs. 1b to 1f): The volume loss (fig. 1b) of the cingulum bundle was calculated from the transversal areas of this structure taken at different positions on the antero-posterior axis of the brain. This parameter was recently (during the previous EEC contract) found to represent **the most sensitive *histological* indicator** of a prenatal irradiation damage **actually** available. Such quotation is not only valid for **the acute** irradiations but **also** for **the protracted** exposures. Although, in the present neutron experiments, the effects found up to now after 7 cGy remain ambiguous (figs. 1b,1e), the 28 cGy were clearly efficient (**-18%**; figs. 1b,1f) and allow to estimate an **RBE of 2.2** with respect to *d14-20pc* gamma rays. This rather small RBE value is provisional: indeed, the present measurements were carried out in the very radiosensitive ***d12-16pc*** "window" and in consequence, the 2.2 RBE may even be overestimated.

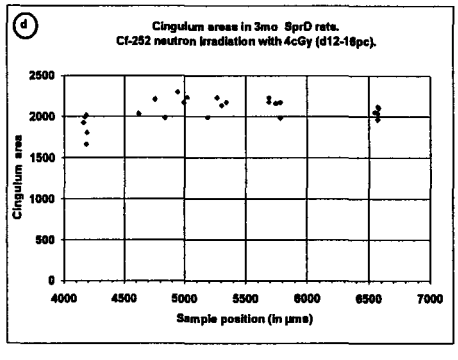
2.- Mechanisms of cingulum involution: The fact that a **continuous** 4-day exposure to ionizing radiations apparently produces the same cingulum atrophy as an **acute** irradiation carried out in a few seconds on day 15 of the pregnancy, casts new lights on the origin of the prenatal damages. Moreover, **in both these modes** of irradiation, the *most sensitive zone of the cingulum* is a 1500 micron long segment in the *anterior* part of the structure (between sampling positions +4500 μms and +6000 μms from the interaural line). However, it is not yet known whether the effects of a **continuous** exposure are more widely *dispersed* over this part. Also, a **difference** between acute and protracted exposures is expected concerning the thin structure of the myelin sheaths which represent the main component of the cingulum.

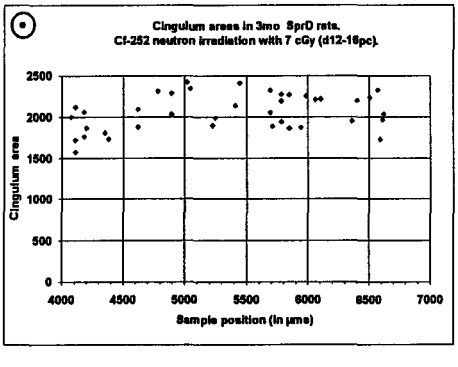
3.- The 7 cGy *cingulum data*(45% observed): The results obtained after 7 cGy (154 mSv) are still incomplete but already reveal a **discrepancy** among the individual responses to a prenatal brain irradiation (fig. 1e): part of the animals behaves as controls but in others, the cingulum is clearly attenuated. Does this **dual** answer reveal the influence of other uncontrolled factors, including the possible phenomenon of *individual radiosensitivity,* an increasingly frequent mention in low doses studies? We don't know. Also, the Sprague-Dawley rats used here were outbred animals, a choice which allows to mimic closely the human situation but which is less adapted to the determination of threshold doses.


a
Brain weight after protracted Cf-252 neutron exposure (d12-16pc). 3mo SprD rats.
Brain Weight (in mg)
1800
1700
1600
1500
1400
1300
1200
0
5
10
15
20
25
30
Total Neutron dose in cGy


b
Cingulum Volume after protracted neutron exposure (d12-16pc). 3mo SprD rats.
Cingulum Volume (in µm3 x 10E 3)
5000
4000
3000
0
10
20
30
Total Neutron Dose (cGy).


c
Cingulum areas in 3mo SprD rats. Controls.
Cingulum area (in µm2)
2500
2000
1500
1000
500
0
4000
4500
5000
5500
6000
6500
7000
Sample position (in µms) along brain main axis


d
Cingulum areas in 3mo SprD rats. Cf-252 neutron irradiation with 4cGy (d12-16pc).
Cingulum area
2500
2000
1500
1000
500
0
4000
4500
5000
5500
6000
6500
7000
Sample position (in µms)


e
Cingulum areas in 3mo SprD rats. Cf-252 neutron irradiation with 7 cGy (d12-16pc).
Cingulum area
2500
2000
1500
1000
500
0
4000
4500
5000
5500
6000
6500
7000
Sample position (in µms)

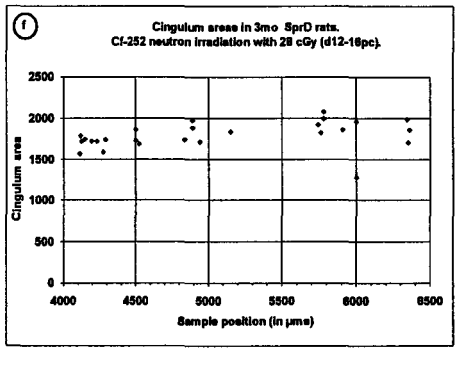

f
Cingulum areas in 3mo SprD rats. Cf-252 neutron irradiation with 28 cGy (d12-16pc).
Cingulum area
2500
2000
1500
1000
500
0
4000
4500
5000
5500
6000
6500
Sample position (in µms)

fig.1

c) Synaptophysin: a marker of the synaptic connexions (task 15% completed).

Part of the CEA rat material was fixed for immunocytochemical studies in an attempt to evaluate the possible **loss** of synaptic connexions in the prenatally irradiated animals. This intent raised not only from the above observations over the **general atrophy** of the brain but also from other data showing a dose related **disorganization of the dendritic arborization** and processes of the cortical neurons (Konermann; Adv. Rad. Biol., 1987).

The tissue samples were searched for the presence of synaptophysin, by means of the avidin-biotin immunocytochemistry; **in the cortex**, *no clear diminution was found* even after 28 cGy of neutrons from d12-16pc. Incidentally, the S-100 protein, a glial marker, also remained unmodified after this same "high dose" treatment.

In the hippocampus, *the synaptic density decreased* in the Ammon's horn (CA3) and in the gyrus dentatus. However, the possible influence of the *sampling area* has not yet been exhaustively assessed for this criterion. Also, the quantitative evaluation will be made more accurate using an immuno-gold approach to be developed shortly.

d) The subsurface cisterns (SSC) , an intraneuronal digital dosimeter. EM observations (task 60% completed):

The subsurface cisterns are organelles exclusively found in neurons (Fig 2). This special form of endoplasmic reticulum is easily spotted at the electron microscope level due to its electron dense and collapsed membranes. The **function** of these organelles is still totally **unknown** although most of the authors assume they are related to the electrophysiology of the neuron. Alternatively, they could represent a step in the **neoformation** and maintenance of the endoplasmic reticulum (Reyners et al., 1977). In coping with this view, they decrease with **age** in the rat brain. Moreover, **the SSC also decreased in size** in the neurons of the cortical layer II in 32 day old Wistar rats **irradiated** with 57 cGy gamma rays from d12-16pc. This assessment reveals that the neuron perikaryon (and not only its

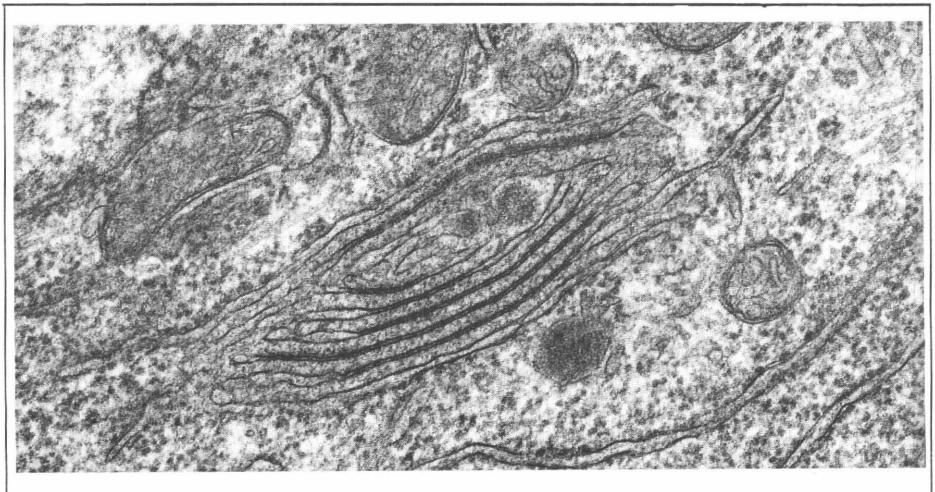


fig 2: A 6-stack subsurface cistern group in a neuronal perikaryon. (Mag = 6.10E5 x).

external processes) bears also the marks of a prenatal irradiation. Unfortunately, the SSC assay is time consuming and was not yet carried out after acute irradiations nor on the present neutron material; its dose limits remain presently unknown.

Protracted exposures and Risk: The neutron irradiation data presented above do largely confirm the occurrence of serious hazards related with a protracted exposure of the fetal brain even to very low doses of irradiation; this rather surprising observation, which was first made with gamma exposures during our participation to the anterior Nuclear Fission Safety program of the EEC (Reyners et al., Int. J. Radiat. Biol., 1992), points out to the limited repair capabilities of the developing brain. Moreover, such an observation is in open contrast with some basic principles in radiobiology, namely the fact that in growing tissues (intestine, regenerating liver), a total restoration after irradiation is only a question of time. So, the response of the brain during the peak of its organogenesis (d12-16pc) is genuine, a situation probably due to the **hierarchized procedure ruling** its development: contrarily to the situation of the other, rather *homogeneous,* organs, the brain is extremely *heterogeneous*; the correct stepwise formation of all its different compartments and nuclei depends not only on a precise time-shedule but also on the previous installment of a series of "inductive" structures at critical places. It is the complexity of this program which makes it sensible. Such a situation could only be partially circumvented in a few areas (Cerebellum, hippocampus; Ferrer, this report).

In conclusion, the present experiments cast new lights not only on the development of the brain but also on its systems of defense. The protracted exposures contribute to reveal and to assess the weaknesses of the repair potential of the developing brain; they also represent a new useful clue towards the final understanding of the extremely high **radiosensitivity** of the developing Central Nervous System.

LIST OF PUBLICATIONS:

In order to avoid unnecessary duplication, the three contractants to this project have regrouped their recent (1992-1993) publications under a unique list to be found at the end of the current document.

The conferences and symposiums where they have presented communications supported by the Nuclear Fission Safety program of the EEC are also mentioned there.

Head of project 2: Dr Coffigny

II. Objectives for the reporting period

- Carrying out ^{252}Cf neutron exposure protracted from 12 to 16 or 16 to 20 days of gestation.
- Selection of the cell development stage in the cortex and the hippocampus which allowed for the largest production of cells in culture.
- Characterisation of the radial glial cells.
- Identification of dividing glial cells after BrdU labelling.
- Establishment of a threshold value and a dose-effect relationship in the granule cells of the dentate gyrus after *in vivo* gamma irradiation of 14 day-old rats (work of F. Ménétrier).

III Objectives for the next reporting period

- Irradiation with a ^{252}Cf source of pregnant rats from day 12 to16 (7 cGy/day) for Reyners, Ferrer and Coffigny experiments.
- Part of cell environment in the response to irradiation. This point will be studied with mesencephalic and striatal cells irradiated either *in vivo* just before cell isolation and culture or just after cells isolation but before to set culture.
- Identification of glial cells and neurones by immunohistochemistry will be carried on.
- Identification of dopamine, acetylcholine and GABA neurones by immunohistochemistry will be carried on.

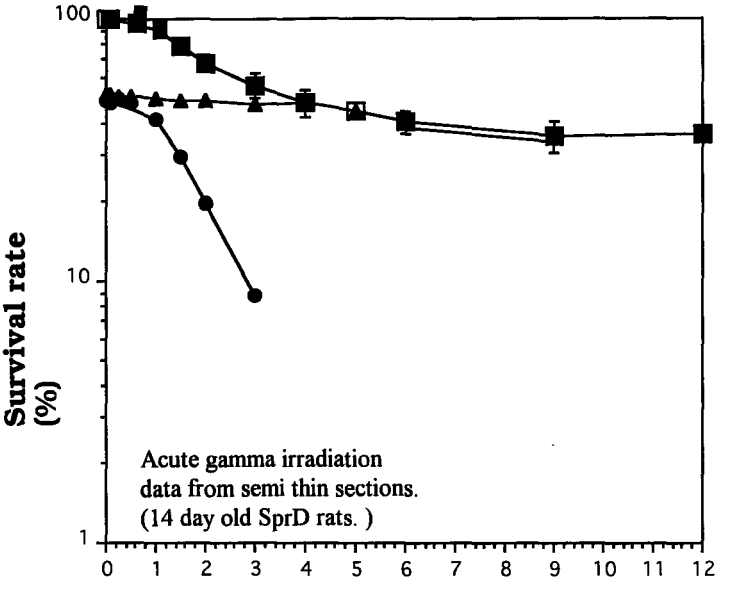


fig. 5 Survival rate as a function of dose delivered at 6 hours after irradiation in the dentate gyrus.

■ Graph corresponding to the two cells populations.

▲ The most radioresistant cells population with a D0 = 22,7 Gy

● The least radioresistant cells population with a D0 = 1,2 Gy

IV Progress achieved

- Two series of protracted ^{252}Cf exposure were carried out:

1) from 12 to 16 or 16 to 20 days of gestation with dose rates of 1 cGy/day and 2.5 cGy/day. Seventy three females born from 18 dams (litter homogenised to 4 females and 4 males) were sacrificed when 90 day-old and their brains were fixed and analysed (Reyners, Mol). The 72 males were studied for another topic (Coffigny).

2) from 12 to 16 days of gestation with dose rates of 1, 1.75 and 7 cGy/day. Seventy six females born from 19 dams were studied as previously (Reyners). Sixteen males (4 litters) and 16 irradiated males with dose rate of 7 cGy/day (4 litters) were sacrificed when 7 day-old. The brains were fixed with two protocols and sections were analised by immunohistochemistry (Ferrer, Barcelona). At the end of exposure, the hippocampus and the cortex cells of control and irradiated fœtuses were cultivated but failed to develop (Coffigny).

- The optimum period to set culture was 18 days of gestation for the hippocampus, 16 and 18 days of gestation for the cortex.
- The radial glial cells were identified with morphological criteria and with vimentine by immunohistochemistry.
- The dividing cells were labelled with a BrdU antibody but the nature of these cells remains to be identified.
- The dose-effect relationship with granule cells in the dentate gyrus showed:

1) a threshold value of 0.5 Gy,

2) an heterogeneous aspect of the dose-effect curve indicating 2 sub populations: a radiosensitive one (D_0=1.2 Gy) and a radioresistant one (D_0=22.7 Gy).

LIST OF PUBLICATIONS:

In order to avoid unnecessary duplication, the three contractants to this project have re-grouped their recent (1992-1993) publications under a unique list to be found at the end of the current document.

Head of the project 3: I. Ferrer

II. Objectives for the reporting period:

1. LAST PERIOD: Study of the mechanisms involved in the radiation-induced cell death during the development of the Central Nervous System in vivo: a comparison with the naturally occurring cell death.
1. Characterization, distribution and sequence of x-ray induced cell death in the cerebral cortex, hippocampus and cerebellum. Vulnerability at different postnatal stages.
2. Cell types involved in X-ray induced cell death: germinal cells, glial (precursors and mature).
3. Vulnerability at different phases of the cell cycle using markers of selected phases.
4. Effects of protein synthesis inhibitors and growth factors in the prevention of the radioinduced cell death.
5. Role of calcium.

2. NEXT PERIOD: Study of the long term effects on the glial cells following X-irradiation at precise postnatal ages.

III. Progress achieved:

Postnatal cell death in the hippocampus: In this report, we will mostly deal with the sequence of X-ray-induced cell death in the hippocampus of the rat at different **postnatal** ages. For this purpose, Sprague-Dawley rats aged 1 or 15 days were irradiated with a single dose of 200 cGy X-rays (2000 mSV) and killed from 3 to 48h afterwards. In rats aged 1 day the number of dying cells rapidly increased in the hippocampal complex with peak values at 6h after irradiation. This was followed by a return to normal values at 48h after irradiation. Early effects were found in the subiculum (SUB), CA1 and CA2 subfields of the hippocampus (fig 1)*. Lesions in the CA3 subfield, dentate gyrus (GD) and hilus (H) appeared later. Dead cells predominated in the stratum pyramidale (pyr) and stratum oriens (or). **Dying cells were almost absent in the stratum moleculare.** X-ray-induced cell death was abolished with a cycloheximide injection given at the time of irradiation, thus indicating that this death is an active process probably mediated by protein synthesis. The injection of nerve growth factor did not preserve nerve cells from dying. This latter finding suggests that the X-ray death is not dependent on nerve growth factor deprivation.

Neuron versus glia: In an attempt to characterize the cell types involved in X-ray-induced cell death, immunocytochemical studies have shown that only a small percentage of dying cells in the hippocampal complex were neurons, whereas most dying cells were germinal cells and glial precursors. Commercial antibodies were used in the present study, together with anti-Hu antibodies which were separated from the total serum in our laboratory. These antibodies recognize early neuroblasts and neurons, and are, therefore, good neuronal markers in the very early stages of maturation.

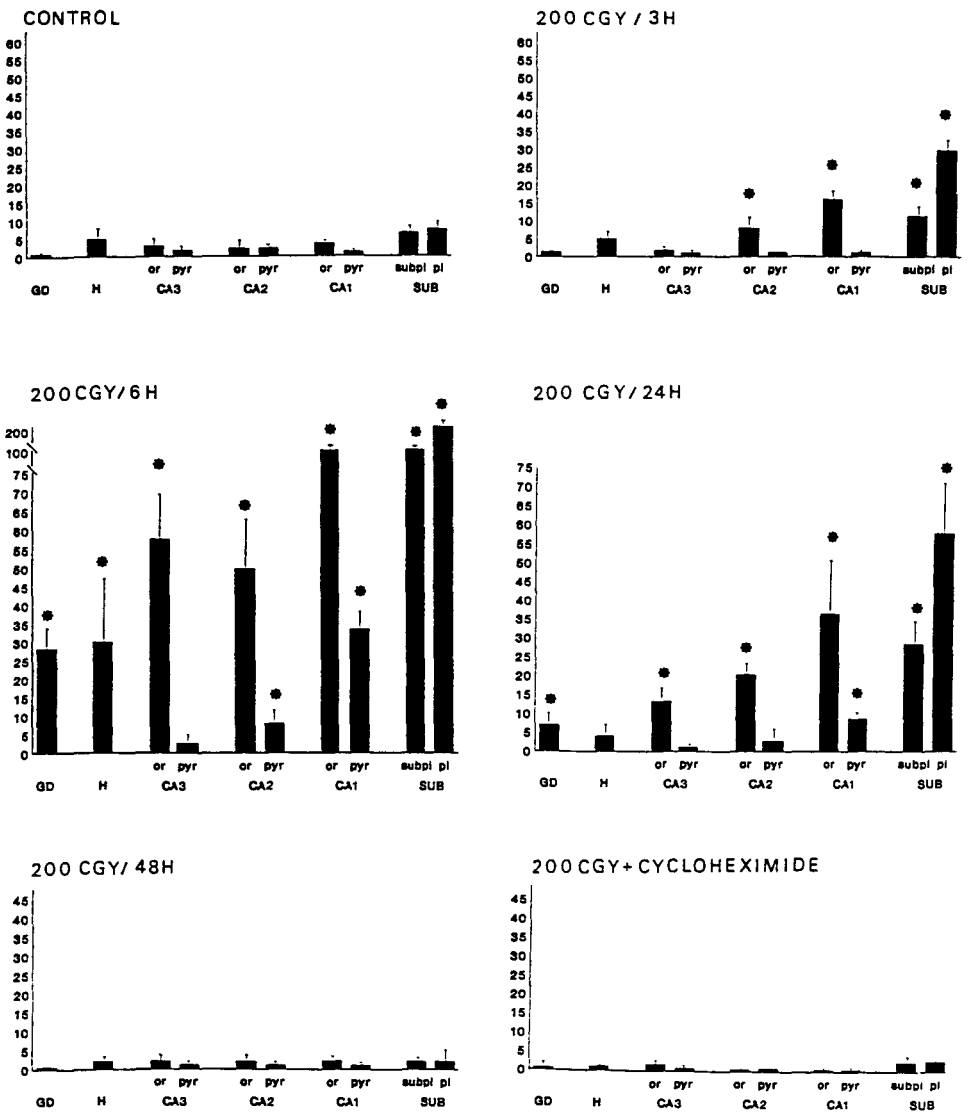


Fig. 4. Number of dying cells (mean values ± SD) in the hippocampal complex in control and 1-day-old X-irradiated rats killed at different intervals (3, 6, 24 and 48 h) after irradiation, and in X-irradiated rats receiving 2 μm/g body weight of cycloheximide at the time of irradiation, and killed 6 h later. GD: dentate gyrus; H: hilus; CA3, CA2, CA1: areas of the hippocampus; SUB: subiculum; or: stratum oriens; pyr: stratum pyramidale; pl: cortical plate (cellular layer); subpl: cortical subplate (future subcortical white matter). Asterisks indicate $p < 0.0001$ in relation to the corresponding values in controls (Mann-Whitney U-test).

fig. 1

In the cerebellum: X-ray-induced cell death was also examined in the developing cerebellum. This structure was chosen because the external granular layer, which is a multipotential germinal layer, is still active in the newborn rat. The cerebellum was also considered in order to study long-term recovery of damaged structures. Rats aged 1 or 3 days were irradiated with 200 cGy X-rays, and the animals were killed at different intervals from 3h to 30 days after irradiation. Peak values of cell death were similar in the cerebellum and hippocampus, and here **again, cycloheximide injection prevented** nerve cells from dying. Immunocytochemical studies disclosed that germinal cells in the external molecular layer (most of them early neuroblasts, according to their immunoreactivity with the anti-Hu antibody), Bergmann cells and glial cells in the white matter were sensitive to X-irradiation. Purkinje cells were not.

Quantitation: The number of dying cells in the external granular layer (EGL), internal granular layer (IGL) and white matter (WM) were measured in animals irradiated at postnatal day 1 (P1) or day 3 (P3). In addition, the stage of the cell cycle in which cells were most vulnerable to X-ray exposure was examined by counting the number of mitoses in EGL, and labelling cells with antibodies against the proliferating cell nuclear antigen (PCNA) which recognizes the S phase of the cell cycle. A marked decrease in the number of PCNA immunoreactive cells was found 6h and 24h after irradiation. This feature, together with the observation of PCNA-immunoreactive dying cells 3h after irradiation indicate that cells in the S phase are most vulnerable to X-irradiation.

In contrast with the Mol observations in the white matter of the brain, the surviving cells in the external granular layer and PCNA-immunoreactive cells in other layers and white matter appear to be able to reconstitute the cerebellum following an acute dose of X-rays.

Taken together, the results accomplished during the reported period, increase our understanding about sensitive cell populations, sensitive phases of the cell cycle, vulnerable developmental periods, and regional and areal patterns of vulnerability of the developing brain following X-irradiation.

The present data are reported in full detail within 2 papers (1993) presented in the final section (III b) of this progress report.

* : This figure corresponds to Fig.4 in J. Neuropathol. Exp. Neurol, 1993.

LIST OF PUBLICATIONS:

Please, see part III b, hereafter.

III B. Progress achieved : Publications.

Coffigny H., Beauvallet M. and L. Court. **Effects of gamma or neutron protracted irradiation during the whole gestation of rat.** XXIV congress of the European Society of Radiobiology. Erfurt (FRG). Book of abstracts. p41, 1992.

Coffigny H., Beauvallet M., Ferrer. I., Reyners H., Gianfelici de Reyners E. and L. Court . **Comparison of the effects of gamma irradiation on rat mesencephalic and striatal nerve cells in culture.** XXVth congress of the European Society of Radiobiology. Stockholm (S). Book of abstracts, p P02:03, 1993. (and in: EULEP Newsletter 72: 22, 1993.)

Ferrer I., Reyners H., Gianfelici de Reyners E. and H. Coffigny. **X-irradiation increases cell death in the cerebral cortex and subcortical white matter of developing rats and its effect is inhibited by cycloheximide.** XXIV congress of the European Society of Radiobiology. Erfurt (FRG). Book of abstracts. p44, 1992.

Ferrer I., Soriano E., Del Rio J.A., Alcantara S. and C. Auladell. **Cell death and removal in the cerebral cortex during development.** Progress in Neurobiology 39: 1-43, 1992.

Ferrer I. **X-ray induced cortical malformation in the rat.** EULEP Newsletter 72:22, 1993.

Ferrer I., Alcantara S. and E. Marti. **A four-layered lissencephalic cortex induced by prenatal X-irradiation in the rat.** Neuropath. Appl. Neurobiol. 19: 74-81, 1993.

Ferrer I., Alcantara S., Zujar M.J. and C. Cinos. **Structure and pathogenesis of cortical nodules induced by prenatal X-irradiation in the rat.** Acta Neuropathol. 85: 205-212, 1993.

Ferrer I., Reyners H., Gianfelici de Reyners E. and H. Coffigny. **Cell vulnerability in x-ray induced apoptosis in the developing cerebral cortex.** XXVth congress of the European Society of Radiobiology. Stockholm (S), Book of abstracts, p P11:04, 1993.

Ferrer I., Santamaria J., Alcantara S., Zujar M.J. and C. Cinos. **Neuronal ectopic masses induced by prenatal irradiation in the rat.** Virchows Archiv A Pathol. Anat. 422:1-6, 1993

Ferrer I., Serrano T., Alcantara S., Tortosa A. and F. Graus. **X-ray induced cell death in the developing hippocampal complex involves neurons and requires protein synthesis.** J. Neuropath. Exp. Neurol. 52:370-378, 1993.

Reyners H., Gianfelici de Reyners E., Coffigny H. and I. Ferrer. **Protracted prenatal exposure with low doses of gamma rays and neutrons: late consequences in the central nervous system.** XXIV congress of the European Society of Radiobiology. Erfurt (FRG). Book of abstracts. p43, 1992.

Reyners H., Coffigny H., Lemaire G., Ferrer I. and E. Gianfelici de Reyners. **La radiosensibilité du cerveau foetal aux faibles doses et aux faibles débits de dose d'irradiation externe.**Colloque "Radiobiologie: Aspect biologiques et bases physicochimiques". Sherbroke (Can), Book of abstracts, p. 84, 1993.

Reyners H., Gianfelici de Reyners E., H. Coffigny and I. Ferrer. **Quantitative histological assessment after low dose rate neutron irradiation during the corticogenesis of the fetal rat brain.** XXVth congress of the European Society of Radiobiology. Stockholm (S). Book of abstracts, p P02:17, 1993.

(Reyners H., Gianfelici de Reyners E. and J.W. Hopewell. **Early vascular indicators of the radiation syndrome in the adult rat brain.** XXVth congress of the European Society of Radiobiology. Stockholm (S). Book of abstracts, p L08:03, 1993.)

Reyners H., van Ravestyn L. and E. Gianfelici de Reyners. **Neurologie du retard mental sévère causé par une irradiation prénatale.** Ann. Ass. Bel. Radioprotection 18,151-157 (1993).

III C. Participations to meetings.

1. **Erfurt** (Germany): 4 October 1992: 24th annual meeting of the Eur.Soc.Rad.Biol.: Session: "Embryology".(contributors: REYNERS, COFFIGNY, FERRER).

2. **Didcot** (UK): 26 October 1992: Eulep workshop on Prenatal Irradiations. (REYNERS, COFFIGNY, FERRER).

3. **Reisensburg** (Germany):8 March 1993: Annual Eulep Meeting. Contributors to G. Konermann report: "RECENT ADVANCES IN PRENATAL RADIOBIOLOGY".(REYNERS, COFFIGNY,FERRER).

4. **Brussels** (Belgium): 19 March 1993: Meeting of the "Société Belge deRadioprotection". Theme:"RADIOSENSIBILITE DE L'EMBRYON". (invited speaker: REYNERS).

5. **Bad Honeff** (Germany): 29 March 1993: EEC contractor meeting. Session:"DEVELOPING ORGANISM, BRAIN, THRESHOLDS". (REYNERS).

6. **Stockholm** (Sweden): 10 June 1993: 25th annual meeting of the Eur.Soc.Rad.Biol.: *1.) Session:*"RADIOSENSITIVITY AND PREDICTIVE ASSAYS". (REYNERS, COFFIGNY). *2.) Session:*"APOPTOSIS". (FERRER).

7. **Sherbroke** (Canada): 12 July 1993: Colloq ue"Radiobiologie et Bases Physicochimiques". (REYNERS, COFFIGNY,FERRER).

Progress Report

Contract: FI3P-CT920064c Sector: B33

Title: Dosimetry and effects of parental, fetal and neonatal exposure to incorporated radionuclides and external radiation.

1)	Harrison	NRPB
2)	Henshaw	Univ. Bristol
3)	Van den Heuvel	VITO
4)	Lord	Inst. Paterson
5)	Visser	TNO - Delft
6)	Tejero	Univ. Madrid - Complutense
7)	Bueren	CIEMAT
8)	Paquet	CEA - Bruyères-le-Châtel

I. Summary of Project Global Objectives and Achievements

I.I Objectives

To undertake the studies detailed in the Project Proposal, following from work previously funded under Contracts B16-347d and B17-002c, including:

1) measurements of natural alpha emitters and $^{239/240}$Pu in human fetal tissues;
2) studies of the transfer of radionuclides, principally ^{210}Po, ^{210}Pb, ^{239}Pu, ^{241}Am, ^{141}Ce and ^{106}Ru, to the embryo and fetus, mainly in rodents;
3) the use of animal and human data to estimate doses to the human fetus and child after maternal intakes of radionuclides;
4) studies of the effects of external irradiation and ^{239}Pu and ^{241}Am contamination on murine haemopoietic development *in utero* and after birth, using cellular and molecular techniques;
5) studies in mice of the effect of paternal exposure to ^{239}Pu or ^{241}Am prior to conception on haemopoietic development in offspring; and
6) studies of *in utero* and neonatal biological effects on the brain in rats after exposure to external irradiation for comparison with effects of bone-seeking radionuclides, initially ^{141}Ce.

I.II Progress achieved

I.II.I Human data

A detailed investigation is being undertaken at UB of the distribution of natural alpha radioactivity within the human fetus, using autopsy samples obtained from various stages of development from 18 weeks to stillborn. Samples from 84 cases have been collected, mainly from the Bristol area but including samples from West Cumbria. Alpha-activity in tissue samples, largely due to ^{210}Po, is being measured using the track detector, TASTRAK. Concentrations in soft tissues have been consistently below the limit of detection of about 50 - 70 mBq kg^{-1} but concentrations in vertebrae, the selected bone sample, range from the detection limit to about 500 mBq kg^{-1}.

The results obtained do not show a difference between samples from Bristol and Cumbria. Further analyses undertaken during the period have been used in comparisons of vertebral activity as a function of gestational age, suggesting a correlation between age and increasing concentrations. Additional data have provided further evidence indicating a correlation between increasing concentration and the proximity of the mothers residence to the Severn estuary. Analysis showed that fetal age was not an

important confounding factor.

Measurements are being made at NRPB of concentrations of ^{210}Po and other alpha-emitters in fetal tissues obtained from second trimester terminations carried out in the Oxford area and in Cumbria. No correlation has been observed between ^{210}Po concentration and either geographical location or maternal age. Ethical Committee approval has been obtained to extend this study to include fetal tissue samples from high ^{222}Rn areas in Cornwall and provision of samples is being pursued. Measurements of $^{239/240}$Pu have also been undertaken for selected samples. The values obtained are on the limit of detection by mass spectrometry but provide valuable information on the low concentrations of Pu in the fetus compared with fetal Po concentrations and maternal Pu concentrations. Measurements on pooled samples are currently being undertaken to improve the reliability of results obtained. It is also intended to collaborate with UB in the measurement of $^{239/240}$Pu in bone samples from still-births.

Collaborative studies have been initiated with Chelsea and Westminster Hospital to measure radionuclide transfer through perfused human placentae. These studies should provide information on the comparative behaviour of different elements including their retention in tissues of the placenta.

I.II.II Animal studies - biokinetics/dosimetry

Previous studies at NRPB of the transfer of the alpha-emitters, ^{210}Po, ^{238}Pu and ^{241}Am, to the embryo and fetus in rats and guinea pigs, together with the available human and primate data, have been used to estimate doses to haemopoietic tissue. The animal data suggest that in each case the main determinants of leukaemogenic risk from *in utero* exposure in humans are likely to be irradiation of the yolk sac in early gestation and bone marrow during the fetal growth period. It was concluded that *in utero* doses to haemopoietic tissue from ^{210}Po will exceed those from ^{239}Pu and ^{241}Am at elevated environmental levels of actinide exposure (eg. near to Sellafield). However, important dosimetric uncertainties remain, primarily concerning the distribution of dose within the irradiated tissues and species differences in transfer particularly during the fetal growth period.

The distribution of alpha dose from ^{239}Pu within fetal tissues, and particularly the yolk sac, is being studied using the beta emitter, ^{241}Pu, to localise the cellular location of the element. Preliminary results with rat yolk sac show association with cell nuclei; the possibility that this may be an artifact due to the movement of Pu during histological processing is being pursued.

In a collaborative study between CEA and NRPB, the transfer of ^{210}Po, ^{239}Pu and other actinides to the fetus in late gestation is being studied in a primate species, the baboon. Results for ^{239}Pu show transfer to be greater than at comparable stages in rats and guinea pigs with similar concentrations in fetal and maternal bone.

In studies at VITO, pregnant mice were given ^{241}Am either by injection on day 14 of pregnancy or by constant infusion from implanted osmotic pumps between days 7 - 14 or days 14 - 19. Pregnancy resulted in higher ^{241}Am concentrations in bone but lower concentrations in the livers of the mothers. Concentrations in neonates at birth were greater in the femur than liver and were highest in each case after infusion in late gestation and lowest after infusion in early gestation, reflecting greater transfer at later stages.

Studies at CEA, designed to estimate doses delivered to fetal tissues and organs during development, involve measurement of the skeletal distribution of different radionuclides with differing energies, and analysis of biological effects. The studies are initially being focused on the uptake of radionuclides by the skull and on biological effects on brain and cerebellum. In particular, apoptosis in the cerebellum is being studied to assess the absorbed dose in this organ.

During the period, the skeletal distribution of ^{141}Ce has been studied in rats after contamination at fetal and neonatal stages since development of the cerebellum continues for a short period after birth. Rats were given ^{141}Ce at different stages of late gestation and distribution determined either one day later or on day 21. Rats were also given ^{141}Ce at different times after birth and in each case groups were killed after 5 h, 24 h and 72 h. Results showed that ^{141}Ce is retained mainly in bone, the skeleton accounting

for 60 - 85% of total fetal or neonatal activity at one day after administration. The proportion of retained activity in the fetal and neonatal liver was in the range 10 - 40% of total body activity. During the fetal period, the liver : skeleton ratio decreased, reaching a minimum at birth and increasing with neonatal age. Other organs, including the brain, did not accumulate significant ^{141}Ce activity. Measurements of the distribution of ^{141}Ce between different bones of the skeleton showed that the ribs and the spinal column both accounted for about 10 - 15% of total skeletal activity. The skull accounted for about 5 - 10% of total activity. Variations with developmental stage were observed which will affect doses to the brain and other tissues.

Measurements have been made of apoptosis in the cerebellum of rats after external irradiation at 20 mGy h^{-1} for 0, 17, 29 or 41h. Significant numbers of apoptotic cells were apparent at the lowest dose and a clear dose response relationship was established. Studies with ^{141}Ce are in progress.

Studies at NRPB are being extended to other radionuclides, currently including ^{106}Ru and ^{144}Ce. Ruthenium as ^{106}Ru citrate was administered to rats at different stages of pregnancy and to guinea pigs either before conception or in late pregnancy. The results for rats showed transfer of up to 0.05% per fetus at birth. On day 12 after administration 3 days previously, activity transferred to the fetus was about 30 times less than to the placenta and yolk sac; the concentration of ^{106}Ru in the fetus was about 0.01% injected activity g^{-1} while the concentration in the yolk sac was about 1 % g^{-1}. The highest concentration in maternal tissues, consistent with published data, was for the kidneys with about 3 % g^{-1}. Transfer to the guinea pig in late gestation was 0.2% of injected activity per fetus after administration 7 days previously and two orders of magnitude lower after administration one month prior to conception. Cerium as ^{144}Ce chloride was administered to rats either before conception or at different stages during pregnancy and to guinea pigs in late gestation. Preliminary results for rats show transfer of up to 0.01% injected activity per fetus shortly before birth. Concentrations in the yolk sac were more than two orders of magnitude greater than in the fetus.

The information obtained in studies of the transfer of radionuclides to the fetus is being used in current ICRP work on the estimation of doses to the human fetus.

I.II.III Effects on haemopoiesis

The sensitivity of haemopoietic tissues to irradiation during development has been demonstrated in experiments using mice at PICR, VITO and CIEMAT. These investigations are continuing and have been extended to consider effects of paternal irradiation prior to conception and effects in young animals.

Studies at CIEMAT have investigated whether irradiation with doses as low as 0.5 Gy (X-rays) will induce persistent haemopoietic damage at particular stages of murine development. Mice have been irradiated on day 4, 13 or 17 of *in utero* development, as neonates or as young adults. After *in utero* irradiation on day 13 or 17, long-term deficiencies were observed in the granulo-macrophage lineage.

Studies have been initiated at NRPB to determine effects in the marrow of neonatal CBA/H mice after *in utero* irradiation, by a combination of cytogenetic and clonogenic techniques. Specific attention will be given to stable chromosomal changes, particularly those having a known association with leukaemogenic transformation. Clonogenic studies will investigate the growth factor dependence of haemopoietic cells after irradiation. During the period, mice have been exposed to 0.5 Gy of X-irradiation at either 7 or 14 days of gestation and analyses of marrow cells are in progress; comparisons with ^{239}Pu exposure will follow.

Studies at VITO have shown haemopoietic damage in mice after *in utero* contamination with ^{241}Am either by injection on day 14 of gestation or infusion in early or late gestation (mothers given 450 Bq g^{-1}; see I.II.II). The dose to the femur in offspring was estimated as 4 to 20 mGy. Changes in the number of haemopoietic progenitor cells (CFU-GM) and a disturbance of the stromal haemopoietic microenvironment were observed in all groups until at least 6 months after birth.

The determination of RBE values for alpha-irradiation is problematic because of the heterogeneity of both alpha-irradiation within tissues and the distribution of appropriate target cells. For *in vivo* effects on haemopoiesis, PICR have chosen to investigate persistent long-term damage to haemopoiesis as the most realistic approach. By this means, direct damage to the stem cells is bypassed and the more relevant response of the overall system is assessed. Previous studies demonstrated that maternal ^{239}Pu contamination results, in the offspring, in a persistent deficit of haemopoietic stem cells which conforms to a specific spatial distribution. These effects have been simulated with external gamma-rays, applied continuously (or in daily acute doses) over the same gestational period. Compared with the effect of ^{239}Pu incorporation giving an estimated dose to the fetal liver of 10-14 mGy, 900 mGy of gamma-irradiation had a discernable effect but at least 3.6 Gy of chronic gamma-irradiation was required to simulate the effect of ^{239}Pu, suggesting an effective RBE of 250 - 360 for this end-point. Acute daily doses of gamma-irradiation were somewhat more effective, with an equivalent dose of about 1.8 Gy, suggesting an RBE of 130 - 180.

Studies at VITO have continued on the effect on haemopoietic and stromal stem cells after paternal contamination of BALB/c mice with ^{241}Am (3, 6, or 30 kBq) prior to conception. No changes in bone marrow cellularity and haemopoietic stem cells (CFU-s, day 14) were seen up to 6 months after birth. Changes in the number of haemopoietic progenitors (CFU-GM) and stromal stem cells (CFU-f) were observed; CFU-f numbers were generally increased. The stromal microenvironment, as evaluated by the capacity to establish *in vitro* long-term bone marrow cultures, appeared to be disturbed.

In parallel studies at PICR, male mice were injected with 64 or 128 Bq g^{-1} ^{239}Pu at one or three months prior to mating with uncontaminated females. Their offspring were assessed individually for haemopoiesis at 6 weeks of age. The overall cellularity and GM-CFC content was normal in all cases but the pluripotent spleen colony-forming units were reduced at the higher doses - this was significant in mice treated 3 months earlier. Compensatory hyperproliferation in the differentiation and maturation stages resulted in a normal output of cells from the stressed progenitor cell compartment. The fibroblastoid colony-forming cells - a component of the regulatory microenvironment - was similarly reduced at the higher doses for both precontamination periods. This may indicate that transmitted genetic damage does not specifically target haemopoiesis per se but effects the development of a competent microenvironment, capable of supporting unstressed haemopoiesis.

Comparisons have been made at VITO of the sensitivity of different haemopoietic tissues to *in vitro* alpha-irradiation from ^{210}Po. Preliminary results for clonal assays for CFU-GM and stromal stem cells (CFU-f)) showed no difference in sensitivity between adult marrow, neonatal marrow, fetal liver (15d) and fetal spleen and marrow (18d).

Methods are being developed at CIEMAT to transfer stable genetic markers to lympho-haemopoietic stem cells through retroviral vectors. The introduction of unique markers into self-renewing stem cells has now been confirmed by following differentiation in secondary and tertiary recipients transplanted with the transduced population. Studies of the effect of radiation on differentiation patterns will now be undertaken.

With the aim of obtaining information relating to the molecular basis of the persistent haemopoietic deficiencies observed after irradiation, analyses of the expression of haemopoietic growth factors (HGFs) have been evaluated, initially in animals irradiated with high doses of X-rays (CIEMAT). Preliminary studies have shown that the haemopoietic microenvironment of long-term bone marrow cultures (LTBMCs) established 1 year after a 7 Gy irradiation is primed to express high amounts of most of the HGFs tested (G-CSF, IL-1, stem cell factor). In collaboration with other Contractors, similar experiments will be carried out after low doses of chronic low-LET external irradiation, or after contamination with alpha-emitting radionuclides.

At UCM, studies have continued on the effect on murine stromal cell metabolism and granulocyte function of long-term X-irradiation (5 Gy). Persistent increases in G6PD activity have been observed in granulocytes from LTBMCs at long times after irradiation. This enhancement was associated with increased production of NADPH connected with phagocytic activity. In addition, protein production was increased. Characterisation of the GM-CSF content of LTBMC supernatants demonstrated overproduction. To confirm the role of GM-CSF in granulocyte function, cells from control animals were

incubated with LTBMC from irradiated animals. Increases in superoxide anion production, G6PD activity and protein levels were observed in these cells. These data confirm that GM-CSF released after irradiation is responsible for observations of enhanced granulocyte functional capacity.

Studies have been initiated on metabolic characteristics of stromal cells in adherent layers from one year after irradiation. Preliminary results show an increase in protein levels as well as G6PD activity in reduced populations, indicative of residual damage. Studies of metabolic characteristics of these cells are in progress.

Measurements at PICR of superoxide-anion production have shown that in all cases where residual damage occurs in haemopoietic tissue - whether from alpha-particle contamination, external X- or gamma-irradiation, or from specific biological deficiencies manipulated *in vitro* - the functional activity of the granulocytes produced is enhanced. The increase appears to be associated with changes in their patterns of growth factor production.

At TNO, haemopoietic stem cells from normal murine bone marrow have been analyzed, by a combination of cell separation techniques and application of the reverse-transciptase polymerase chain reaction, to investigate the presence of mRNA for growth factor receptors. The modulation of the expression of genes encoding for these receptors and for adhesion molecules will give novel information about the regulation of haemopoiesis at the stem cell level.

The analysis and sorting of the haemopoietic stem cells was performed by combining density gradient centrifugation and FACS sorting. cDNA libraries were made of the different subpopulations and partial sequencing revealed new genes, some of which seemed stem cell specific. Six new cdc2-related genes were discovered in the stem cell cDNA bank as well as three known ones. The expression of the alpha-unit of the GM-CSF receptor was found to be upregulated concomitantly with the differentiation of the pluripotent stem cells. The alpha-unit of the interleukin 3 receptor was found to be expressed both in pluripotent and committed stem cells. Since the pluripotent stem cells do not readily respond to IL-3, in contrast to the committed progenitors, this suggests that the IL-3 receptor beta-unit is upregulated when the pluripotent stem cells differentiate. Among the receptors that were found to be expressed, there were several for which no ligand is known. This indicates that interesting new growth factors or stem cell specific inhibitors may be found in the near future. These first results with the cDNA banks indicate that interesting differences between stem cell fractions can be elucidated by these procedures. Strategies for the selection of a limited number of genes for detailed further studies are now being developed.

In studies at CIEMAT of methods to ameliorate the effect of radiation exposure on the haemopoietic system, polysaccharide molecules capable of stimulating murine haemopoietic precursors have been investigated for their ability to mediate the endogenous production of HGFs and confer radiation protection *in vivo*. Some of these molecules have been shown capable of enhancing the expression of G-CSF, M-CSF, GM-CSF and IL-1 in murine LTBMCs. In some cases, *in vivo* treatment of mice with these molecules showed evidence of a radiation protection effect. These molecules will also be tested in LTBMCs established with human bone marrow. In studies of *ex vivo* expansion of bone marrow, transplantation after expansion with IL-1 and IL-6 has been shown to improve haemopoietic regeneration in irradiated recipients.

Current studies at PICR with TNF-alpha have confirmed in *in vitro* radioprotective properties. These results, however, were not supported by *in vivo* treatments with TNF-alpha. Final high dose, *in vivo* studies are currently underway to check this discrepancy.

I.III Objectives for next reporting period

CIEMAT will continue studies on the radiosensitivity of haemopoietic stem cells at different stages of development and the molecular basis of observed changes, on studies using genetically labelled stem cells and on methods of counteracting haemopoietic damage.

Studies at PICR are comparing the effect of alpha-emitters and external radiation on haemopoiesis for exposure at different stages of development including paternal exposure. Changes to haemopoietic

progenitor cells and their regulatory microenvironment are being pursued. Studies will include assessment of the potential of alpha-particles to cause *in vitro* transformation of growth factor dependent progenitors into factor independent cells.

VITO will continue with studies on long-term marrow cultures from mice after paternal exposure to ^{241}Am or maternal exposure; growth factor production will be studied. *In vitro* alpha-irradiation using ^{210}Po is being used to assess the radiosensitivity of haemopoietic progenitor cells and stromal cells from the bone marrow and liver of mice at different developmental stages.

UCM are extending their studies of long-term radiation effects to different stages of development. LTBMCs from mice exposed at different times after birth will be evaluated during the next period and mice have been irradiated at 4 and 13 days of gestation (1 or 3 Gy) for further studies.

TNO will further investigate the expression of genes encoding for growth factor receptors or adhesion molecules in different subpopulations of haemopoietic stem cells.

Having established apoptosis in the cerebellum of rats as a sensitive indicator of dose from external radiation and studied the distribution of ^{141}Ce in the fetus and neonates, studies at CEA will focus on apoptotic yield after ^{141}Ce contamination. The aim is to establish the internal dose absorbed by the cerebellum after internal contamination. Comparisons with ^{85}Sr and ^{238}Pu are also planned.

UB will continue with analyses of concentrations of ^{210}Po in human fetal bone samples from different regions and will use the data to estimate doses.

Depending on the availability of tissue samples, NRPB will measure concentrations of natural alpha emitters in human fetal tissues from Cornwall (high radon area) and $^{239/240}$Pu concentrations in fetal bone samples collected by UB. Studies of the fetal transfer of radionuclides in rodents and collaborative studies with CEA of transfer in the baboon will be continued. Experiments initiated to determine bone marrow damage in offspring of mice by cytogenetic and clonogenic analyses after *in utero* irradiation will be extended from the effect of X-irradiation to contamination with ^{239}Pu.

I.IV Contractors meeting

A meeting was held 8-10th December 1992 at GSF-Neuherberg, jointly with groups on "Induction of osteosarcoma and leukaemia by bone-seeking, alpha-emitting radionuclides" and "Cytogenetic and molecular mechanisms of radiation myeloid leukaemogenesis in the mouse".

Head of project 1: Dr. Harrison

II. Objectives for the reporting period

To undertake the following studies:
1) measurements of natural alpha emitters and $^{239/240}Pu$ in human fetal tissues;
2) studies of the transfer of radionuclides, principally ^{210}Po, ^{210}Pb, ^{239}Pu, ^{241}Am, ^{141}Ce and ^{106}Ru, to the embryo and fetus, mainly in rodents;
3) continuation of collaborative studies with CEA on the transfer of ^{210}Po, ^{237}Np, ^{239}Pu and ^{241}Am to the baboon fetus;
4) the use of animal and human data to estimate doses to the human fetus and child after maternal intakes of radionuclides;
5) studies of the effects of *in utero* exposure to external irradiation on murine haemopoietic development after birth, using clonogenic and cytogenetic techniques for subsequent comparison with effects of ^{239}Pu and other nuclides.

III. Progress achieved including publications

III.1 Human data

Measurements have been made of the concentration of Pu in human fetal tissue obtained from second trimester terminations carried out in the Oxford area and in Cumbria. These determinations have been undertaken using standard chemical separation techniques and sensitive detection systems, such as alpha spectrometry and thermal ionisation mass spectrometry. Typical fetal tissue concentrations of a few $\mu Bq\ kg^{-1}$ were recorded; placental concentrations were of the same order of magnitude. These concentrations are approximately two orders of magnitude lower than those typically found in tissues of adult members of the UK population. No differences were observed in concentrations of Pu in fetal tissue obtained from West Cumbria and Oxfordshire. Therefore, it would appear that the enhanced levels of Pu in the West Cumbrian environment, as a result of discharges from the Sellafield nuclear fuel reprocessing plant, do not result in a measurable increase in Pu concentrations in fetuses of pregnant females from this area. Measurements on pooled samples are currently being undertaken to improve the reliability of results obtained. It is also intended to collaborate with UB in the measurement of $^{235/240}Pu$ in bone samples from still-births.

Measurements have also been made of concentrations of naturally-occurring radionuclides in human fetal tissues and their distribution between fetus and placenta. Measurements of ^{210}Pb, ^{210}Po, ^{238}U and ^{232}Th in tissues from Oxfordshire are complete; analyses on samples from Cumbria are continuing.
Results obtained show similar concentrations of ^{210}Pb and ^{210}Po at 4 - 60 $mBq\ kg^{-1}$ for fetal tissue and 8 to 140 $mBq\ kg^{-1}$ for placental tissue. Corresponding values for ^{238}U and ^{232}Th were in the range 0.1 to 0.9 $mBq\ kg^{-1}$. The results do not show any correlation between ^{210}Po concentration and either

geographical location or maternal age. Ethical Committee approval has been obtained to extend this study to include fetal tissue samples from high ^{222}Rn areas in Cornwall and provision of samples is being pursued.

Collaborative studies have been initiated with Chelsea and Westminster Hospital to measure radionuclide transfer through perfused human placentae. These studies should provide information on the comparative behaviour of different elements including their retention in tissues of the placenta.

III.II Animal studies - biokinetics/dosimetry

Previous studies at NRPB on the transfer of the alpha-emitters, ^{210}Po, ^{238}Pu and ^{241}Am, to the embryo and fetus in rats and guinea pigs, together with the available human and primate data, have been used to estimate doses to haemopoietic tissue. The animal data suggest that in each case the main determinants of leukaemogenic risk from *in utero* exposure in humans are likely to be irradiation of the yolk sac in early gestation and bone marrow during the fetal growth period. It was concluded that *in utero* doses to haemopoietic tissue from ^{210}Po will exceed those from ^{238}Pu and ^{241}Am at elevated environmental levels of actinide exposure (eg. near to Sellafield). However, important dosimetric uncertainties remain, primarily concerning the distribution of dose within the irradiated tissues and species differences in transfer, particularly during the fetal growth period.

The distribution of alpha dose from ^{239}Pu within fetal tissues, and particularly the yolk sac, is being studied using the beta emitter, ^{241}Pu, to determine the cellular location of the element. Preliminary results with rat yolk sac show association with cell nuclei; the possibility that this may be an artifact due to the movement of Pu during histological processing is being pursued.

In a collaborative study between NRPB and CEA, the transfer of ^{210}Po, ^{239}Pu and other actinides to the fetus in late gestation is being studied in a primate species, the baboon. For measurements on one mother and fetus at 7 days after administration at 5 months, the fetus accounted for 4% of administered ^{239}Pu and about 0.4% of ^{241}Am, with corresponding retention in the placenta of 11% for ^{239}Pu and 2% for ^{241}Am. For ^{239}Pu, the results show greater fetal : maternal concentration ratios (C_F/C_M ratios) than obtained with rats and guinea pigs. Further collaborative work is in progress.

Studies are being extended to other radionuclides, currently including ^{106}Ru and ^{144}Ce. The information obtained in these studies is being used in current ICRP work on the estimation of doses to the human fetus.

Ruthenium as ^{106}Ru in citrate solution was administered to rats at different stages of pregnancy and to guinea pigs either before conception or in late pregnancy. The results for rats showed that transfer measured at 3 days after administration increased from about 0.0002% of injected activity per fetus on day 12 of gestation to about 0.05% at birth. The corresponding C_F/C_M concentration ratios were about 0.08 and 0.1. For transfer on day 12 of gestation, the retention of ^{106}Ru by each fetoplacental unit was about 0.2% of administered activity, with 40% of transferred activity in the decidua, 30% in the yolk sac, 30% in the placenta and 1% in the fetus. Concentrations were greatest in the yolk sac at about 1% g^{-1} compared with 0.01% g^{-1} in the fetus.

Transfer to the guinea pig in late gestation at 7 days after administration (days 50 - 57) was 0.2% injected activity per fetus corresponding to a C_F/C_M ratio of 0.2. Transfer to each fetoplacental unit was 2% of injected ^{106}Ru with 50% of transferred activity retained in the yolk sac, 35% in the placenta and 10% in the fetus. Total retention in three to four fetoplacental unit was therefore 6 - 8% compared with 14% in other tissues of the mother. Concentrations were greatest for the yolk sac at 0.3% g^{-1}, with values of 0.04% g^{-1} for the placenta and 0.004% g^{-1} for the fetus. For administration 4 weeks prior to conception, the level of ^{106}Ru retained in the fetus on day 57 of gestation was two orders of magnitude lower than after short-term administration with a C_F/C_M ratio of about 0.004.

Cerium as ^{144}Ce chloride was administered to rats either before conception or at different stages during pregnancy and to guinea pigs in late gestation. Preliminary results for rats show that transfer measured 3 days after administration increased from about 3.10^{-5}% injected activity per fetus on day 12 to 0.001% on

day 18 and 0.01% shortly before birth. The corresponding C_F/C_M ratio increased from 0.004 to 0.02. For transfer measured on day 12, the retention of ^{144}Ce by each fetoplacental unit was about 0.001% of injected activity, with 60% of incorporated activity in the decidua, 5% in the yolk sac, 30% in the placenta and 2% in the fetus. Concentrations were greatest in the yolk sac at about 1% g^{-1} compared with 0.002% g^{-1} for the fetus. For transfer shortly before birth, the retention of ^{144}Ce by each fetoplacental unit was 0.2% of injected activity, with 33% of incorporated activity in the decidua, 36% in the yolk sac, 20% in the placenta and 9% in the fetus. Concentrations in the yolk sac, placenta and decidua were 0.5% g^{-1}, 0.1% g^{-1} and 0.4% g^{-1}, respectively, compared with 0.003% g^{-1} in the fetus.

III.III Effects on haemopoiesis

Studies have been initiated at NRPB to determine effects in the marrow of neonatal CBA/H mice after *in utero* irradiation, by a combination of cytogenetic and clonogenic techniques. Specific attention will be given to stable chromosomal changes, particularly those having a known association with leukaemogenic transformation. Clonogenic studies will investigate the growth factor dependence of haemopoietic cells after irradiation.

During the period, mice have been exposed to X-irradiation (0.5 Gy) at either 7 or 14 days of gestation and analyses of marrow cells are in progress; comparisons with ^{239}Pu exposure will follow. Preliminary results after X-irradiation suggest that progenitor cell numbers in neonates shortly after birth may be reduced.

III.IV Publications

Bradley EG and Prosser SL (1993) Radionuclides in human fetal tissues. Radiol. Prot. Bull. (in press).

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Stather, JW, Harrison JD and Kendall, GM (1992) Radiation doses to the embryo and fetus following intakes of radionuclides by the mother. Proc. CEC/USDOE Workshop on Age-Dependent Factors in the Biokinetics and Dosimetry of Radionuclides, Nov. 1991. Radiat. Prot. Dosim. 41, 11-118.

Stather, JW, Harrison, JD and Kendall, GM (1992) Uptake and distribution of radionuclides in the embryo and fetus - implications for dosimetry. In: Radiation Research - A 20th Century Perspective (edited by Dewey, WC, et al) Proc. 9th ICRR, Toronto, July 1991. Academic Press, Toronto. Vol II, pp. 306-311.

Head of project 2: Dr. Henshaw

II. Objectives for the reporting period

There is uncertainty about the transfer of radioactivity from mother to fetus. The aim of this study is to obtain information on the absolute levels of alpha-activity within the fetus as a function of fetal development in order to calculate the radiation dose to cells at risk. Also information is needed on transplacental transfer so that fundamental mechanisms of transfer from the mother can be elucidated.

III. Progress achieved including publications

The objective is to obtain this information by using plastic track detectors to analyze autopsy fetal samples for total alpha-activity. To date fetal tissue samples from 84 cases have been collected and stored against TASTRAK alpha-particle detecting plastic. To date 32 cases have been fully analyzed, while the rest remain under storage in the deep freeze to accrue sufficient track accounts for analysis. A summary is given in Table 1.

Table 1 Table to show numbers of fetal spine samples currently under investigation

	Total number samples	Total number fetal spine samples	Number of fetal spine samples scanned	Number of fetal spine samples in storage	Number of fetal spine awaiting scanning
Southmead, Bristol samples	69	64	28	36	0
Cumbria samples	15	7	4	3	0

The detection limit for the technique employed is in the range 50-70 mBq kg^{-1} and the activity in soft fetal tissues is typically below this value. However, activity in the fetal skeleton has been shown to be readily detectable with measured values ranging up to 0.5 Bq kg^{-1}. In order to model fetal transfer of alpha-activity, two corrections need to be applied to the values inferred from the autoradiographs: (i) for ingrowth of ^{210}Po from ^{210}Pb in the fetus, and (ii) for similar ingrowth on the autoradiograph itself.

Measurements carried out to date have provided first results of the absolute alpha-activity levels in fetal spine and the variation with fetal age. Figure 1 shows a histogram of measured values where the terms IS and OS refer to the inner and outer sections of vertebrae respectively. Examination of the activity concentration values for outer vertebrae show that this increases with fetal age as indicated in Figure 2. The rate of increase is presumed to follow the rate of calcification of fetal bone. It is of interest that the intercept at zero concentration occurs at a gestational age of ~0.45 or 18 (range 9-23) weeks, and this is presumably related to the age of formation of the fetal skeleton.

The analysis of an initial set of samples showed evidence of an apparent correlation between alpha-activity from ^{210}Po in fetal spine and distance of mothers residence from the Severn Estuary near Bristol. A further independent set of nine samples showed a similar trend. The variation of activity with proximity with the Severn Estuary for the total data is shown in Figure 3. These data have been examined in more detail and the results are summarised in Table 2. Thus the correlation coefficient r for the second data set is 0.54 but this is not statistically significant, $p = 0.1$, but for the total data $r = 0.59$ which is statistically significant with $p < 0.001$.

In view of the significant variation of vertebrae activity with age of fetus we have examined the possibility that this is a confounding factor in producing the correlation with proximity to the Severn Estuary. In Figure 3 the fetal age has been reliably estimated for 17 of the cases, but the association for this set is still significant: $r = 0.66$; $p < 0.005$. When these data are normalised to a fetal vertebrae activity at 32 weeks gestation using the best fit in Figure 2, the correlation is again significant: $r = 0.71$; $p < 0.001$. Therefore, the effect of fetal age has not confounded the data and the correlation of fetal vertebrae activity with proximity to the Severn Estuary remains.

This evidence of an apparent association of activity with proximity to the Severn Estuary is most unexpected and we have no ready explanation for the effect. Published work on ^{210}Pb intake by inhalation suggests that up to 30% of ^{210}Pb in the bloodstream of the mother could arise from direct inhalation of ^{210}Pb. However, it is difficult to envisage such wide variations in airborne ^{210}Pb levels that could account for the observed effect in the fetus.

A number of fetal cases have been analyzed from West Cumbria, although samples of vertebrae were obtained for only one of these. In all soft tissues no activity was detected and the activity values in vertebrae were similar to that obtained in Bristol samples. The results are summarised in Table 3.

Table 2. Summary of data sets exhibiting correlation between a-activity in fetal vertebrae and proximity to Severn Estuary.

Data Set	Number of cases	Correlation Coefficient	Significance
1992/3 (fig 5)	9	0.54	p=0.1
All data (fig 6)	26	0.59	p<0.001
Fetal age known (fig 7)	17	0.66	p<0.005
Normalised to 32 weeks (fig 8)	17	0.71	p<0.001

Table 3: Summary of alpha-activity in fetal tissues from Cumbria (ND = Not detected)

Case No.	Tissue	Activity Bq/Kg
S74	Umbilical cord	ND
"	Placenta	ND
S75	Placenta	ND
"	Umbilical cord OS	ND
"	Umbilical cord IS	ND
S83	Thymus	ND
"	Placenta	ND
"	Placenta	ND
"	Vertebral column IS	0·219 ±0·08
"	Vertebral column OS	ND
"	Vertebral column IS	0·210±0·09
"	Vertebral column OS	0·313±0·09
"	Thymus	ND
"	Spleen	ND
"	Spleen	ND

Figure 1. Total activity on α-autoradiographs of fetal vertebrae.

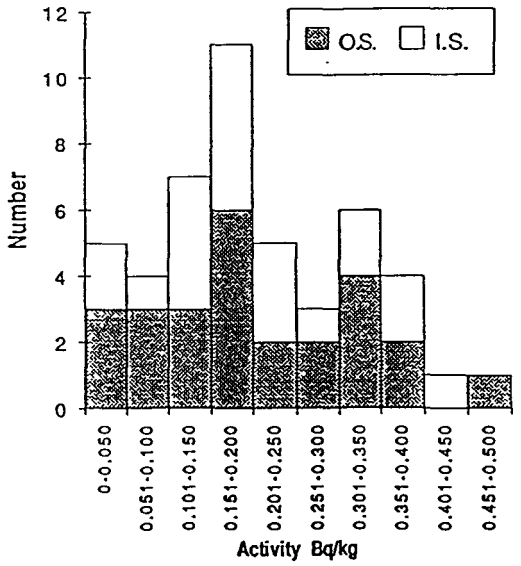


Figure 2. Fetal vertebrae outer activity with respect to age.

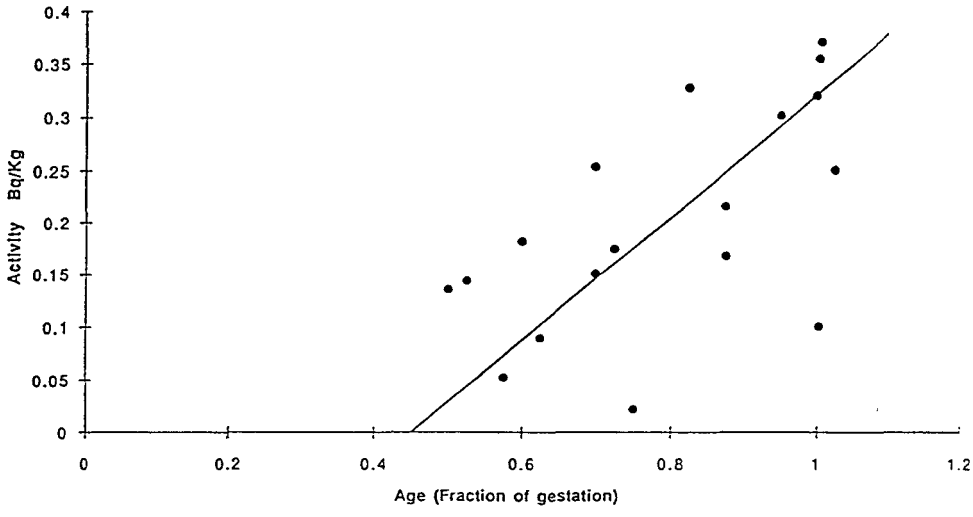
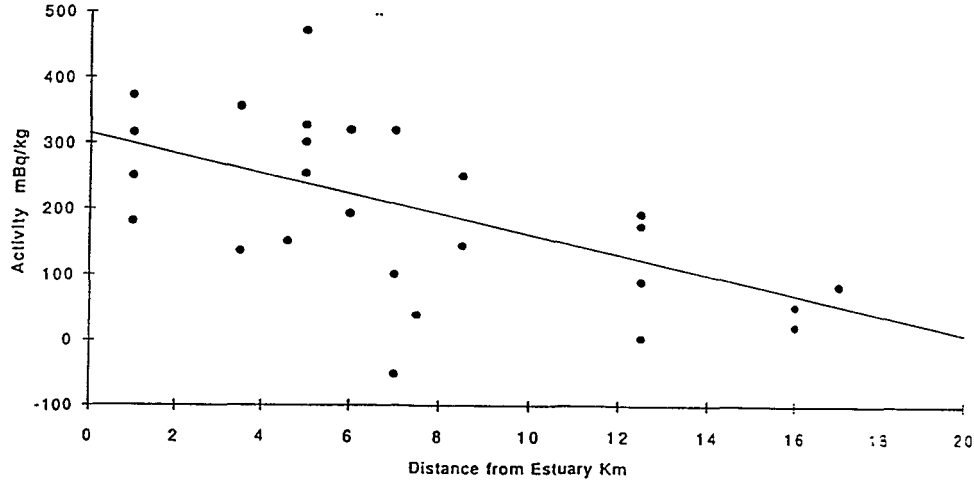


Figure 3. Fetal vertebrae activity vs distance from sea of mother's residence - All data



Head of project 3: Dr. Van den Heuvel

II. Objectives for the reporting period

1) To determine radiosensitive stages during pre- and post-natal development for both the response of stromal and haemopoietic marrow cells. Besides maternal contamination (acute and chronic administration of ^{241}Am), special attention is given to the effect of paternal contamination on the offspring. Effects of *in vitro* exposure of haemopoietic tissues at different developmental ages to alpha-particle irradiation from ^{210}Po is studied. To search for sensitive parameters to detect radiation damage. As a first approach, studies were focused on growth factor production.

2) To estimate the average radiation dose in the femur of the offspring by Am uptake and metabolism studies following acute (single injection) and chronic (osmotic pump implantation) radiocontamination of the dams.

III. Progress achieved including publications

Pregnant BALB/c mice and age and sex matched nulliparous controls were contaminated with ^{241}Am (13 kBq per mouse) by two different procedures: (1) a single intraperitoneal injection at the 14th day of gestation, and (2) continuous administration between the 7th and 14th day of gestation and between the 14th and 19th day of gestation. Continuous administration was achieved by implanting osmotic pumps filled with ^{241}Am which released the ^{241}Am activity at a constant rate. Five days after the termination of contamination, ^{241}Am incorporation was measured in the tissues of adults and in the liver and the femur of newborn and one-month-old mice. Pregnancy results in higher ^{241}Am concentrations in bone but lower concentrations in the liver of the mothers. Protracted administration of ^{241}Am compared to a single injection resulted in a lower concentration of ^{241}Am in the livers of pregnant mice, their nulliparous controls and from newborn mice. The higher ^{241}Am concentration in the femur at birth after protracted exposure after 14 days of gestation compared to protracted exposure before 14 days of gestation could reflect the increased placental transfer of ^{241}Am with advancing gestational age. Radiation doses to the femur were estimated as between 4 and 20 mGy. Changes in the number of haemopoietic progenitor cells (CFU-CM) and a disturbance of the stromal haemopoietic micro-environment were noticed at these dose levels in all groups until at least 6 months after birth.

Haemopoietic and stromal stem cell studies were continued in offspring after parental contamination before conception (3, 6, 30 kBq to the father). No changes in bone marrow cellularity and haemopoietic stem cells (CFU-day 14) were seen up to 6 months after birth. Changes in the number of haemopoietic progenitors (CFU-GM) and stromal stem cells (CFU-f) were noticed. Increases in the latter populations were most frequently seen. The stromal microenvironment, as evaluated by their capacity to establish *in vitro* long-term bone marrow cultures, seemed to be disturbed. The results (CFU-GM output) after

recharging the stromal layers with total bone marrow and more purified haemopoietic stem cell populations are somewhat equivocal at the moment.

The sensitivity of different haemopoietic tissues (adult bone marrow, neonatal bone marrow, bone marrow and spleen from 18-day-old fetus and fetal liver of 15 days) to *in vitro* α-irradiation from ^{210}Po was compared. ^{210}Po (0. 27. 55. 110. 220 Bq/ml) was added to the tissue culture medium of short-term clonal assays for haemopoietic progenitors (CFU-GM) and stromal stem cells (CFU-f). Preliminary results indicated no difference in radiosensitivity to α-particle irradiation of the fetal and neonatal tissues compared to adult bone marrow.

In our search for sensitive parameters related to residual radiation damage, the supernatant of long-term cultures derived from offspring after maternal or paternal ^{241}Am contamination was checked for the presence of CSA (colony stimulating activity) using a CFU-GM assay. No or only very low CSF activities were noticed. No differences between control and contaminated groups were seen.

Publications

Van Den Heuvel, R, Vander Plaetse, F, Leppens. H, and Schoeters, G. ^{241}Am distribution and retention in pregnant mice, in their offspring and in non-pregnant mice: comparisons between continuous Am administration and single injection. Radiat. Prot. Dosim., 41, 137-142 (1992).

Van Den Heuvel, R. Effects on the bone marrow in offspring of ^{241}Am contaminated mice. Eulep Newsletter, 67, 5 (1992).

Van Den Heuvel, R. Effects of radioactive irradiation on fetal and young organism. Annalen van de Belgische Vereniging voor stralingsbescherming, 17, 193-212 (1992).

Head of project 4: Dr. Lord

II. Objectives for the reporting period

Six lines of work have been addressed during the last 12 months:

(i) Deriving from the Gardner report, an experimental assessment of the effects of paternal plutonium-239 contamination on the development of haemopoiesis in the offspring was undertaken. This was to include assessment of both the haemopoietic progenitor cells and of their regulatory microenvironment.

(ii) To determine the effective RBE of α-particle irradiation on the development and establishment of long-term haemopoiesis following contamination by transplacental incorporation of plutonium-239.

(iii) To assess the long-term functional activity of granulocytes following α-particle contamination *in utero* or low LET external radiation.

(iv) To investigate the radioprotective properties of TNF-α with a view to resolving discrepancies in current *in vivo/in vitro* experimentation.

(v) To establish an assessment of the potential of α-particles (using an external α-source) to cause direct *in vitro* transformation of growth factor dependent progenitor cells into factor independent growth.

(vi) To establish a definitive assay for megakaryocyte/platelet progenitors in order to investigate long-term damage and recovery prospects.

III. Progress achieved including publications

We have demonstrated a dose-dependent deficiency in mice whose fathers had been contaminated with plutonium-239. Damage appeared to be expressed primarily in the regulatory microenvironment. In the latest experiments where the paternal contamination was escalated to 256 Bq g^{-1}, the offspring marrow appeared unable to generate a new microenvironment. Compensatory proliferation in the haemopoietic tissue, however, continued to maintain blood production.

Studies comparing external gamma-irradiation during pregnancy with maternal plutonium contamination showed that the estimated α-particle dose had to be increased by a factor of 250-360 times for gamma-rays in order to generate the same degree of long-term haemopoietic deficiency in offspring. This is also more than 10-fold higher than the recommended α-quality factor and therefore has significant dosimetric implications.

Measurement of superoxide-anion production has shown that in all cases where residual stromal damage occurs in haemopoietic tissue - whether from α-particle contamination, external X- or gamma-irradiation, or from specific biological deficiencies manipulated *in vitro* - the functional activity of the granulocytes produced is enhanced. The increase appeared to be associated with changes in the patterns of growth factor production.

Current studies with TNF-α confirmed its *in vitro* radioprotective properties. These results, however, were not supported by *in vivo* treatments with TNF-α. Final high dose, *in vivo* studies are currently underway to check this discrepancy.

Megakaryocyte colony-forming cell assays have been standardised and preliminary results show that over the medium term (up to 30 d) following external gamma-irradiation, Meg-CFC in the marrow do not recover to control levels. Some compensatory overshoot arises in the spleen.

Delays on the manufacture of the α-irradiation unit have prevented any direct experimental but considerable preparatory groundwork has been covered and work on this aspect of the project should now commence in the immediate future.

Publications

Mason, T M, Lord, B I, Molineux, G, and Humphreys, E R (1992). Alpha-irradiation of haemopoietic tissue in pre- and post-natal mice: 2. Effects of mid-term contamination with ^{239}Pu *in utero*. Int. J. Radiat. Biol., 61, 393-403.

Lord, B I, Mason, E R, and Humphreys, E R (1992). Age-dependent uptake and retention of ^{239}Pu: Its relationship to haemopoietic damage. Radiat. Prot. Dosim., 41, 163-167.

Gaitan, S, Tejero, C, Humphreys, E T, and Lord, B I (1993). A relationship between residual stromal damage in haemopoietic tissue and the functional activity of granulocytes. Exp. Hematol. In press.

Jiang, T-N, Lord, B I, and Hendry, J H (1993). Alpha-particles are extremely damaging to developing haemopoiesis. Radiation Res. In press.

Head of project 5: Dr. Visser

II. Objectives for the reporting period

Haemopoietic stem cells of normal bone marrow and fetal liver will be analyzed and sorted by a combination of cell separation techniques, RT-PCR and culture methods. The analysis and sorting of the haemopoietic stem cells will be performed by combining density gradient centrifugation and FACs sorting for wheat-germ agglutinin-positive, monoclonal antibody 15-1.1 negative cells with medium forward and low perpendicular light scatter intensity. Sub-populations of this cell fraction will be sorted using Rhodamine 123 and a new antibody directed against the product of c-kit. The different sub-populations will be compared with respect to their short-term and their long-term repopulating ability *in vivo* by transplantation studies at TNO, whereas these molecular characteristics will be analyzed by reverse transcriptase-polymerase chain reaction with cDNA of the sorted fractions. Fluorescent *in situ* hybridisation methods will be developed to detect which cells in the stromal layers are producing biological response modifiers, haemopoietic growth factors and inhibitors, and to what extent that production can be damaged by radiation. In a parallel project adhesion molecules will be analyzed with respect to their role in the interaction between stem cells and stromal layers by employing PCR of sorted haemopoietic stem cells cDNA.

III. Progress achieved including publications

Haemopoietic stem cells of normal mouse bone marrow were analyzed and sorted by a combination of cell separation techniques and application of the reverse-transcriptase polymerase chain reaction to investigate the presence of mRNA for growth factor receptors. In addition, the presence of the receptors was studied using biotinylated growth factors and monoclonal antibodies directed against specific receptors. The modulation of the expression of genes encoding for these receptors and for adhesion molecules will give novel molecular information about the regulation of haemopoiesis at the stem cell level.

The analysis and sorting of the haemopoietic stem cells was performed by a combination of density gradient centrifugation and FACS sorting for wheat-germ agglutinin-positive, monoclonal antibody 15-1.1 negative cells with medium forward and low perpendicular light scatter intensity. Sub-populations of this cell fraction were sorted using Rhodamine 123. cDNA libraries were made of the different sub-populations and these were subtracted and compared. Partial sequencing revealed new genes, some of which seemed stem cell specific when the clones were hybridised to the original stem cell banks as well as to cDNA banks prepared from sorted and activated lymphocytes and from macrophages. Six new cdc2-related genes were discovered in the stem cell cDNA bank as well as three known ones. The expression of the alpha-unit of the GM-CSF receptor was found to be upregulated concomitantly with the differentiation of the plutipotent stem cell. The alpha-unit of the interleukin 3 receptor was found to be expressed both in plutipotent and committed stem cells. Since the pluripotent stem cells do not readily respond to Il-3, in contrast with the committed progenitors, this suggests that the Il-3 receptor beta-unit is upregulated when the pluripotent stem cells differentiate. The product of c-kit, the receptor for Stem Cell Factor (SCF), was found to be equally expressed in the Rh-123 dull and bright fractions.

Also the anti-body ACK-2 bound similarly to these two fractions. However, the biotinylated SCF could be shown to bind to the Rh-123 fraction only. This indicates that the expression of c-kit alone is not sufficient to provide a functional receptor for SCF. Amongst the receptors that were found to be expressed there were several for which no ligand is known. This indicates that interesting new growth factors or stem cell specific inhibitors may be found in the near future.

These first results with the cDNA banks indicate that interesting differences between stem cell fractions can be discovered by this procedure. Strategies for the selection of a limited number of genes for detailed further studies are now being developed.

Publications

Watt, S M, and Visser, J W M. Recent advances in the growth and isolation of primitive human haemopoietic progenitor cells. Cell Proliferation (1992), 25, 263-297.

Ershler, M A, Nagorskaya, T V, Visser, J W M, and Belyavsky, A V. Novel CDC2-related protein kinases in murine haemopoietic stem cells. Gene (1993), 124, 305-306.

DeJong, M O, Rozemuller, H, Visser, J W M, and Bauman, J G J. A sensitive method to detect cell surface receptors using biotinylated growth factors. Progress in Histochemistry and Cytochemistry (1992), 26(N1-4), 119-123.

Corso, A, Hogeweg-Platenburg, M G C, De Vries, P, and Visser, J W M. A protocol for the enrichment of different types of CFU-S from mouse fetal liver, Haematologica (1993), 78, 5-11.

Visser, J W M, Rozemuller, H, DeJong, M O, and Belyavsky, A. The expression of cytokine receptors by purified haemopoietic stem cells. Stem Cells (1993), 11(suppl 2), 49-55/

Visser, J W M, and Hogeweg-Platenburg, M G C. Identification and isolation of bone marrow stem cells. In: Flow Cytometry, NATO ASI Series Vol. H 67 (Jacquemin-Sablon, A., ed) (1993). pp 141-154. Springer-Verlag Berlin, Heidelberg.

Head of project 6: Dr. Tejero

II. Objectives for the reporting period

The objectives for the present period were to extend investigations initiated in the last contract, with respect to granulocyte functionality from long-term irradiation mice (5 Gy X-rays).

Since the increase of superoxide anion by granulocyte NADPH-oxidase system, reported in the last report, may be related to Glucose 6-phosphate dehydrogenase (G6PD) activity, studies in this enzyme have been performed in granulocytes from X-rays irradiated mice.

Characterize the excess of CSFs release after 6 and 12 months post-irradiation, and study the role of these haemopoietic growth factors in granulocyte functionality.

With the aim of evaluating the microenvironmental damage after X-rays radiation, analyses have been undertaken of some parameters in stromal cells long-term after irradiation.

In order to extend our studies of long-term radiation effect to different developmental ages, mice at 4 and 13 days of gestation have been irradiated with 1 and 3 Gy (1 Gy/min) for later studies on granulocyte functionality.

III. Progress achieved including publications

Methodology

Mice C57B1 x BALB/c females (12 weeks old) were total body irradiated with 5 Gy X-rays at a dose rate of 1.03 Gy/min. At day 4 and 13 of embryo development mice were irradiated with 1 and 3 Gy (1.03 Gy/min).

At 6, 9 and 12 months post-irradiation, LTBMCs were established. Cultures were fed weekly and mature granulocytes subsequently assayed.

To test for GM-CSF in LTBMC, supernatant from the media from irradiated mice were incubated with 48 µgof rat antimouse GM-CSF monoclonal antibody (Genzyme). Neutralised samples were tested for bone marrow GM-CFC growth.

Granulocytes incubated with LTBMC supernatants from irradiated animals were subsequently assayed for superoxide anion production and G6PD activity.

Continuous spectrophotometric assay of SOD-inhibitable reduction of ferrictyochrome x was employed to determine $O^{\cdot}_2$ production. Samples were stimulated with 1 µgTPA.

G6PD activity was determined spectrophotometrically according to the method of Battistuzzi et al. The enzymatic extract contained 0.02-0.1 mg of protein.

Results

Granulocyte function

Granulocytes from LTBMC of 6 months post-irradiated animals showed a significant increase in G6PD activity (Figure 1) with respect to control animals between 3 and 7 weeks culture. This enhancement is associated with increased production of NADPH to perform phagocytosis. Furthermore, the ratio mg protein/10^6 cells at 6 months post-irradiation and several weeks of culture, showed an enhancement about 3.5 fold with respect to control (Figure 2), suggesting an increase in proteins expression. Both results are also observed at 9 months post-irradiation.

Characterisation of LTBMC supernatants

Previous results demonstrated that LTBMC cell-free supernatants were capable of stimulating GM-CFC growth. With the aim of characterising the activity of these supernatants, neutralisation experiments with anti mGM-CSF have been performed. A total inhibition of activity with 48 µganti mGN-CSF per ml of supernatant was observed. These data demonstrate that total body irradiation with 5 Gy (X-rays) causes an overproduction of GM-CSF.

Role of GM-CSF in granulocyte function

With the aim of confirming whether GM-CSF released after irradiation is due to the priming granulocytes, cells from control animals were incubated with supernatants from irradiated mice. These cells showed an increase in superoxide anion production (Figure 3), G6PD activity (Figure 4) and about 1.5 fold of protein levels. Superoxide anion production was similar to those obtained when cells were incubated with 16 ng of mouse rGM-CSF (Figure 3). These results indicate that the overproduction of GM-CSF by radiation primes granulocytes promoting modifications in the non-specific host defence mechanisms.

Stromal cells

Adherent layers from one year post-irradiation cultures were less populated than age-matched control ones. However, the ratio mg protein/10^6 cells was higher in adherent cells harvested from LTBMC of irradiated mice. These results were indicative of residual injury in stroma as a consequence of total body irradiation with 5 Gy. Studies related to some metabolic parameters of these cells are in progress.

Publications

Gaitan, S, Cuenllas, E, Sancho, P, Bueren, J A, and Tejero, C (1992). Mechanisms towards compensation of long-term haemopoietic injury in mice after 5 Gy irradiation: *In vivo* and *in vitro* enhancement of superoxide anion production by granulocytes. Bioscience Reports, 12, 281-292.

Escribano, S, Cuenllas, E, Gaitan, S, and Tejero, C (1992). Overproduction of GM-CSF after total body irradiation with 5 Gy (X-rays). Enhancement of G6PD activity in mice granulocytes. 24th Annual Meeting of the European Society for Radiation Biology. Erfurt, Germany. Abs. 182.

Cuenllas, E, Escribano, S, Gaitan, S, and Tejero, C (1992). Prime mice granulocytes after total body irradiation with 5 Gy X-rays. 38th Harden Conference of the Biochemical Society. Ashford, Kent, p 54.

Grande, T. Gaitan, S, Tejero, C, and Bueren, J A (1993). Residual haemopoietic damage in adult and 8-day old mice exposed to 7 Gy of X-rays. Int. J. Radiat. Biol., 63, 59-67.

Gaitan, S, Tejero, C, Humphreys, E R, and Lord, B I (1993). A relationship between residual stromal damage in haemopoietic tissue and the functional activity of granulocytes. Exp. Haematol., 21, 1227-1232.

Escribano, S, Gaitan, S, Cuenllas, E, and Tejero, C (1993). Granulocyte-macrophage colony stimulating factor released after mice total body irradiation: its role in granulocyte function. 22nd Annual Meeting of the International Society for Experimental Haematology. Rotterdam, The Netherlands. Abs. 497. Exp. Haematol., 21, 1145.

Cuenllas, E, Escribano, S, Gaitan, S, and Tejero, C (1993). Daño residual radioinducido en el estroma hematopoyético de radón: parámetros metabólicos. V Congreso de Investigación sobre el Cancer. Las Palmas de Gran Canaria, Spain. 26-29 Septiembre.

Figure 1.

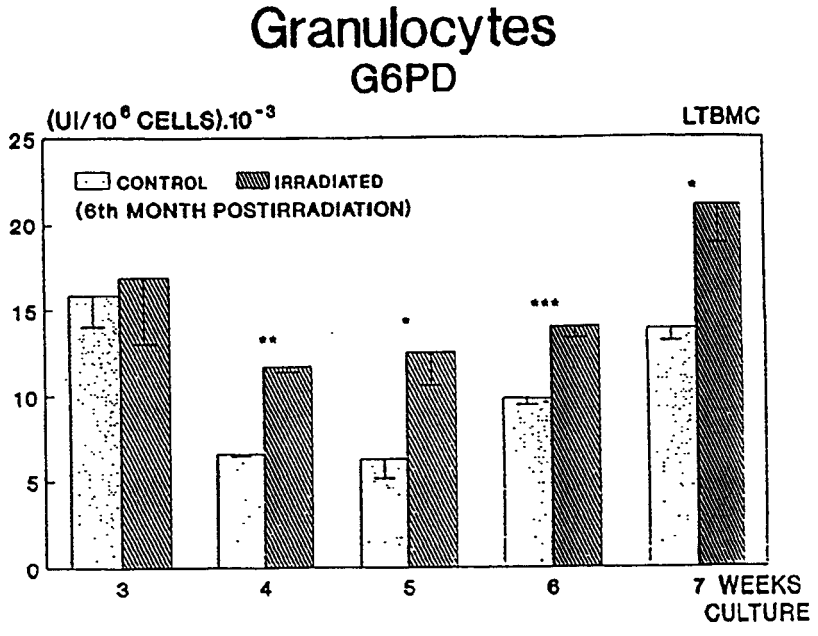


Figure 2.

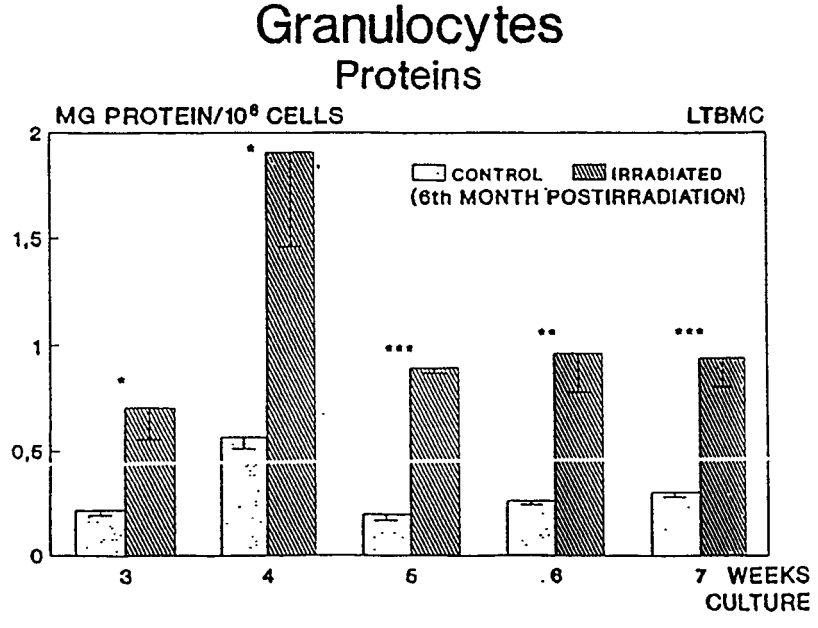


Figure 3.

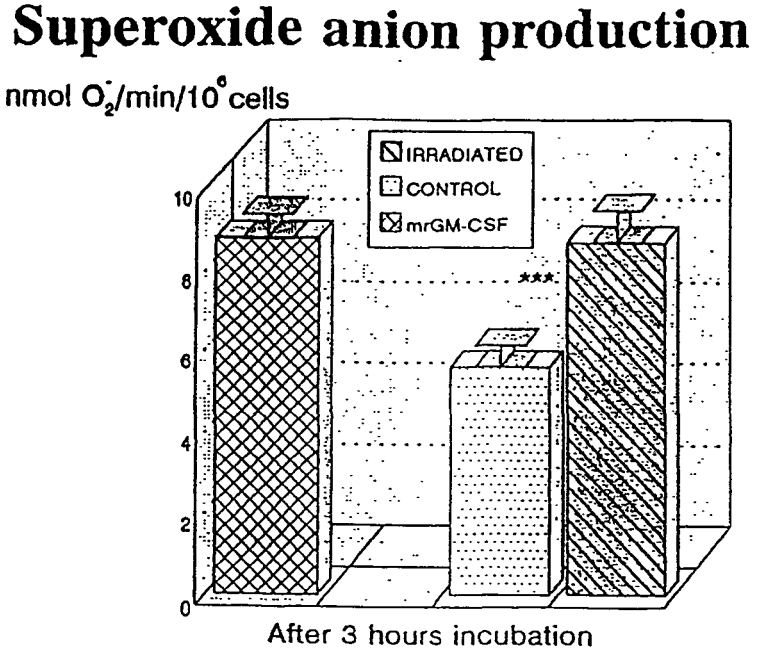
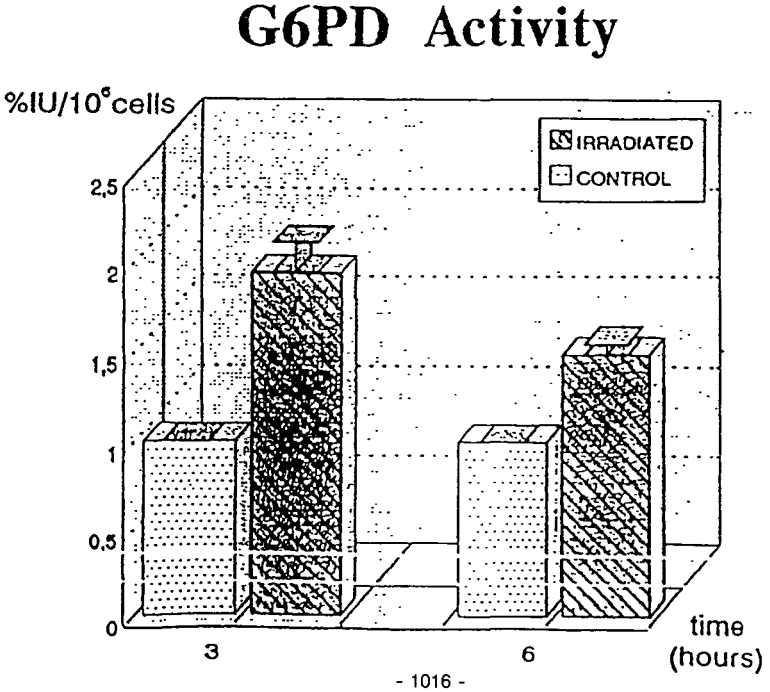


Figure 4.



Head of project 7: Dr. Bueren

II. Objectives for the reporting period

1) To conclude a one-year analysis of clonogenic haematopoietic progenitors present in mice irradiated with 0.5 Gy during early stages of development.

2) To follow the fate of haematopoietic stem cells genetically labelled, following re-transplantation of bone marrow transduced with retroviral vectors.

3) To initiate the investigation on the expression of Haematopoietic Growth Factors in irradiated organisms.

4) To evaluate the expression of Haematopoietic Growth Factors in Long-Term Bone Marrow Cultures treated with polyssaccharides and analyze the haematopoietic recovery in irradiated mice transplanted with *ex vivo* expanded bone marrow.

III. Progress achieved including publications

In order to investigate whether irradiation at particular stages of the mouse development with doses as low as 0.5 Gy (X-rays) induces persistent haematopoietic failures, mice were irradiated at the 4th, 13th and 17th day of embryo development, as well as 2 and 8 days after birth and in the young-adult stage. In our previous report, a significant reduction in the number of CFU-GM was observed 9 months after irradiation at the 17th day of embryonic development. Studies carried out during this reporting period confirmed the reduction in CFU-GM corresponding to these animals at one year post-irradiation. Animals irradiated at the 13th day of embryonic development also manifested a similar reduction one year after irradiation, always in comparison to age-matched control mice. No changes in the CFU-GM population were observed through this period in mice irradiated at the 4th day of embryo development, nor in mice that were irradiated after birth.

In the last reporting period the introduction of genetic labels into primitive haematopoietic precursors was verified by Southern analyses of DNA extracted from the haematopoietic organs of recipients, 10 weeks after transplantation with the transduced marrow. During this reporting period, the introduction of unique gene marks into self-renewing stem cells was confirmed by following their differentiation fate in secondary and tertiary recipients transplanted with the transduced population. The persistence of the genetic label allows us to initiate the study of the differentiation pattern of the haematopoietic stem cells

in the presence and absence of irradiation.

With the aim of obtaining information concerning the molecular basis of the persistent haematopoietic failures observed after irradiation, analyses of the expression of Haematopoietic Growth Factors (HGFs) have been initially evaluated in animals irradiated with high doses of X-rays. Preliminary experiments of Northern Blotting have revealed that the haematopoietic microenvironment of Long-Term Bone Marrow Cultures (LTBMCs) established 1 year after a 7 Gy irradiation is primed to express high amounts of most of the HGFs tested (G-CSF, M-CSF, IL-1, Stem Cell Factor).

In order to ameliorate the haematopoietic syndrome induced by radiation exposures, polysaccharide compounds capable of stimulating murine haematopoietic precursors have been checked for their ability to mediate the endogenous production of HGFs and confer radiation protection *in vivo*. Some of these molecules have been shown to be capable of enhancing the expression of G-CSF, M-CSF, GM-CSF and IL-1 in murine LTBMCs. In some instances the *in vivo* treatment with these molecules showed an evident radiation protection activity in the mouse model.

In experiments involving strategies of *ex vivo* expansion of bone marrow, the transplantation of *ex vivo* expanded bone marrow cells with ILs 3 and 6 has been shown to improve, with respect to conventional bone marrow transplantation, recovery from the haematopoietic syndrome associated to irradiation.

Publications

Grande, T, Gaitan, S, Tejero, C, and Bueren, J A. Residual haematopoietic damage in adult and 8-day old mice exposed to 7 Gy of X-rays (1993). Int. J. Radiat. Biol., 63, 59-67.

Gaitan, S, Cuenllas, E, Sancho, P, Bueren, J A, and Tejero, C. Mechanisms towards compensation of long-term haemopoietic injury in mice after 5 Gy irradiation: *In vivo* and *in vitro* enhancement of superoxide anion production by granulocytes (1992). Biosci. Rep., 12, 281-291.

Bernad, A, Varas, F, Gallego, J M, Almendral, J M, and Beuren, J A. *Ex vivo* expansion and selection of transduced bone marrow: An efficient methodology for gene-transfer to murine lympho-haematopoietic stem cells. Submitted.

Serrano, F, Varas, F, Bernad, A, and Beuren, J A. The *ex vivo* expansion of murine bone marrow grafts with Interleukins 3 and 6 accelerates the haematopoietic regeneration in irradiated recipients without apparent impairment to long-term engraftment. Submitted.

Meetings

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Analysis of the murine haematopoietic engraftment following transplantation of ex vivo expanded bone marrow (1993). Varas, F, Serrano, F, Bernad, A, and Beuren, J A. 8th Symposium of Molecular Biology of Haematopoiesis. Basel.

Analysis of the self-renewal capacity of CFU-S precursors long-term after 7 GY irradiation. Grande, T, and Bueren, J A (1993). 22nd Meeting of the Int. Soc. for Exp. Haematol. Rotterdam.

An improved selection method for retroviral gene transfer to long-term repopulating haematopoietic stem cells (1993). Bernad, A, Varas, F, Gallego, J M, Almendral, J M, and Beuren, J A. Gene Therapy. Keystone Symposia. Colorado.

Effect of AM218, a polycarboxilic, on peripheral blood cells recovery in potentially lethally irradiated mice (1993). Albella, B, Real, A, Guenechea, Bueren. J A, and Maganto. 22nd Meeting of the Int. Soc. for Exp. Haematol. Rotterdam.

Head of project 8: Dr. Paquet

II. Objectives for the reporting period

The objectives for the period from July 1992 to August 1993 were to define the skeletal distribution of 141-Ce in rats after contamination at both embryonic and juvenile stages. The choice of the stages of contamination was supported by the fact that development of the cerebellum continues for a short period after birth. In addition, an attempt was made to measure apoptosis in the cerebellum after external irradiation. These preliminary experiments were necessary to define a dose/effect relationship between apoptosis and the absorbed dose.

III. Progress achieved including publications

1) Skeletal distribution of 141-Ce

Methodology

Two modes of contamination were used, corresponding to different developmental stages in rats.

Firstly, pregnant female Sprague Dawley rats were contaminated at different stages of gestation, from day 17 pc to day 21 (ie, E17, E18, E19, E20). For each stage, three animals were killed 24 hours after contamination and three at day 21. The embryos were dissected and the main organs and skeletal pieces were removed for radiological measurements by gamma counting and autoradiographic studies.

Secondly, juvenile rats were used and contaminated at 1, 3, 5, 7 or 9 days after birth. For each stage, four animals were sacrificed at T = 5 h; T + 24 h and T + 72 h. The dissections and radioactivity measurement were the same as described above.

Results

Figures 1 and 2 show the organ distribution of the radionuclide 24 hours after contamination.

The distribution of 141-Ce occurs mainly in the skeleton. This part of the body accumulates 60 to 85% of the total radioactivity. The liver of the embryos/juveniles accumulates between 10 to 40% of the total radioactivity, depending on the stage of contamination. During the fetal period the ratio liver/skeleton decreases when the development of the fetus increases. The minima was observed at the day of birth. In the same way the ratio increases with the juvenile development, ie when the time elapsed from birth increases. Other organs, like kidneys or brain, do not accumulate cerium significantly. The study of the distribution into the different parts of the skeleton shows that the ribs and spinal column concentrate each between 10 and 15% of the skeletal radioactivity, depending on the stage of contamination. The skull takes account for 5 to 10% and the two femora for approximately 3%. A large part of radioactivity is included in other parts of the skeleton which were not separated (mandible, tibia, humerus, etc).

Discussion

The distribution of 141-Ce in the different organs of rats and in the different parts of the skeleton depends on the developmental stage of the animal when contaminated. The skeleton accumulates Ce

preferentially during the late stages of fetal development, corresponding to the calcification period. Within the skeleton, the skull pays an important role, as a source for irradiation of the brain during development. This part of the skeleton accumulates 5 to 10% of the skeletal radioactivity (ie, 3 to 8% of the total radioactivity) and has a great concentration ability (% IA / g). This localisation of the radionuclide can lead to morphological changes in the brain or in the cerebellum during development. For this reason, it could be interesting to evaluate the microscopical alterations induced, like apoptosis, and to try to correlate them with the radiation dose absorbed.

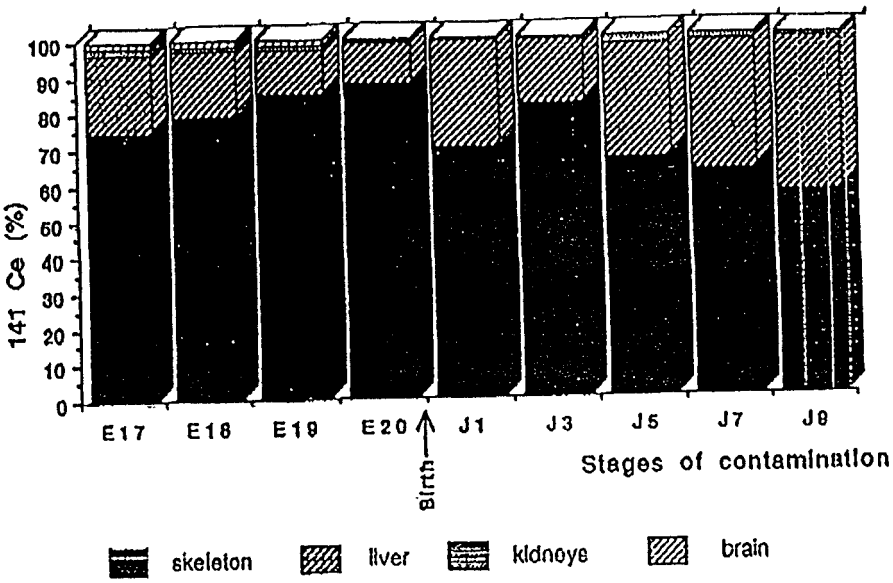
2) Apoptotic studies

Some preliminary experiments were undertaken to define the relationship between the radiation dose absorbed and apoptosis recorded in the cerebellum. Sixteen rats were exposed to chronic external irradiation at a dose rate of 2 cGy/h. The animals were then sacrificed at 0, 17, 20 or 41 hours after the beginning of the irradiation, and the cerebellum was removed and fixed in glutaraldehyde for microscopic alterations studies. Apoptosis was expressed as a percentage of apoptotic cells in the cerebellum.

The results are presented in Figure 3 and show that apoptosis was a very sensitive parameter; 34 cGy delivered at a dose rate of 2 cGy/h was enough to induce apoptosis in a significant manner. When the total absorbed dose was increased, the total apoptotic response was increased.

The next step of the experiments will be to assess the internal irradiation (resulting from internal contamination) by apoptotic studies. Rats will be contaminated with 141-Ce and apoptotic response will be investigated (works in progress). These experiments should permit us to establish the internal dose absorbed by the cerebellum after internal contamination. Further experiments with other radionuclides (85-Sr and 238-Pu) are planned.

Figure 1.



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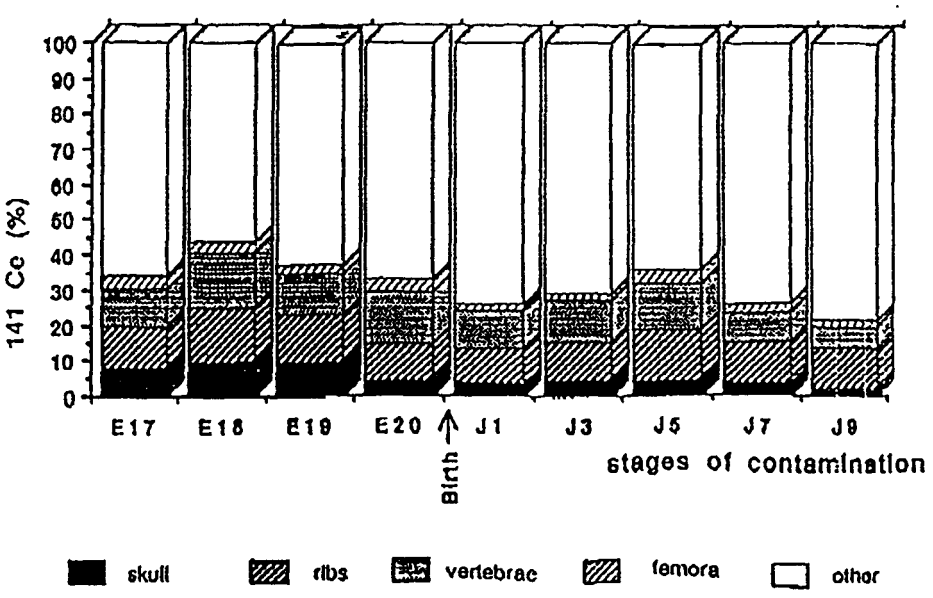

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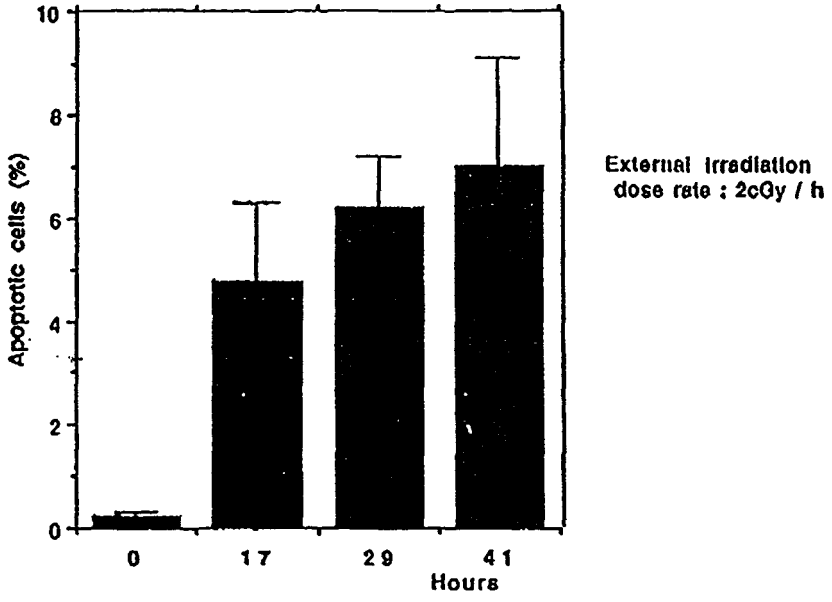

III C

RISIKEN DER STRAHLENEXPOSITION UND IHRE BEWÄLTIGUNG

RISKS AND MANAGEMENT OF RADIATION PROTECTION

RISQUES ET GESTION DE L'EXPOSITION AUX RAYONNEMENTS

Progress Report

Contract: **FI3P-C1920025** **Sector: C12**

Title: Retrospective assessment of radon exposure from long-lived decay products.

1) Vanmarcke	CEN/SCK Brussels
2) McLaughlin	Univ. College Dublin
3) Falk	SSI Stockholm
4) Poffijn	Univ. Gent
5) Samuelsson	Lund University

I. Summary of Project and Global Objectives

The objectives of the RARE (Retrospective Assessment of Radon Exposure) project are to study the chain of processes which in the indoor environment leads from airborne radon to trapped long-lived daughters (LRnDs) and to reveal those exposure conditions in which the trapped activity concentration is a useful estimate of the lung cancer risk caused by radon exposure.

In the first phase of the RARE project (EC Contract No. B17-CT90-0013) it was shown that an analysis of implanted Po-210 into household glass surfaces gives the necessary sensitivity and specificity. In the renewed project the major emphazise will still rest upon implanted LRnDs into surfaces (i.e. surface traps) but, as a complement, also LRnDs trapped by radon diffusion (i.e. volume traps) will be studied.

Surface traps in form of Po-210 implanted into glass sheets are specific to airborne radon daughter activity and have a great potential in large retrospective studies due to high availability of samples of long time exposures and the possibility of using large-scale and non-destructive *in-situ* methods for evaluating the samples. Volume traps have the advantage of being pure radon gas monitors with little or no influence from radon daughter plateout phenomena.

Local variations of plateout rate and the conditions behind the variations will be studied both in radon chambers and in homes. These plateout investigations are important since local variations in plateout can severely degrade the correlation between radon exposure and implanted activity, especially in "one-object-per-house" investigations.

Considering local variations of plateout *in-situ*, the development of large-scale and cheap detectors for implanted Po-210 will be an important part of the project. Different autoradiograhic track-etch techniques will be tested in the laboratory and will be used as a complementary method in epidemiological studies. As in the initial part of the study the open pulse ionisation chamber (PIC) will be used as reference detector of implanted Po-210 into glass sheets. Improvements to the PIC design will be tested during the project period. By means of reference glass samples intercalibration between the contractors and external laboratories of both pulse ionisation chambers and autoradiographic track-etch devices will be performed.

Head of project 1 : Dr. Vanmarcke

II. Objectives for the reporting period

Investigations of the ^{210}Po - activity inside spongy materials due to radon exposure, e.g. mattresses and cushions, as a long-term radon monitor :

1. Exposure of different spongy materials to air with a known radon concentration.

2. Measurement of the ^{210}Po activity in the material accumulated during the exposure and determination of a calibration factor for the average radon concentration.

3. Investigation of the dependence of the calibration factor on the specific properties of the exposed material, e.g. density.

III. Progress achieved including publications

Samples of recently produced synthetic material (polyester) were selected with different densities ρ and stiffnesses κ as listed in table 1. The selection appeared under the considerations that the material should being frequently used in living areas and households and practically being free of any background contribution of ^{226}Ra and ^{210}Po. The material was cut in samples with a volume of about 6 l (10 x 30 x 20 cm^3). During each run four samples of different properties were exposed simultaneously to radon in a chamber with a volume V_o = 95.2 l. The radon concentration was monitored continuously by a lucas-cell. The experimental setup is shown in figure 1. The average exposure was about 30 kBq/m^3y over a period of 30 days. From the half life of ^{222}Rn ($T_{½,\ ^{222}Rn}$ = 3.82 d) and an estimate of its diffusion coefficient ($D_{^{222}Rn} = 2 \times 10^{-6}\ m^2/s$) we may expect the same radon concentration in the material as in the radon chamber. Thus the ^{210}Pb activity of the sample should be of the order of a few Bq.

Table 1. Characteristic properties of the investigated polyester samples. The error on these values is reported by the producer to be less than 10 %.

No of sample	Density ρ (kg/m^3)	stiffness κ (kPa)
1	20.3	2 8
2	29.3	3 8
3	40.1	2.9
4	40 3	4 5
5	44.1	3.1

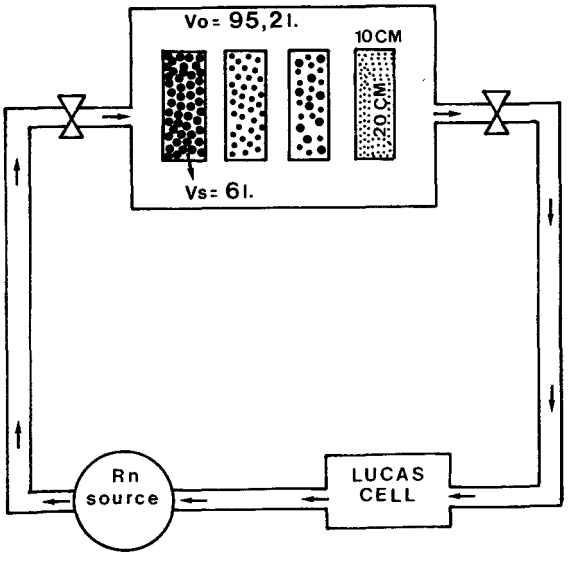

Fig. 1. Schematic representation of the experimental setup used for the exposure of spongy material (polyester) to radon.

After reaching equilibrium activity ($t \approx T_{½,\ ^{210}Po}$ = 138 d) determination of the ^{210}Po - concentration in the material will start. ^{210}Po will be seperated by solving the sample in an acid solution and its activity will be determined by low-background α-spectrometry. The optimization of the chemical procedure is actually under investigation.

An attempt will be made to explain the deposition characteristics, which influence the calibration factor, in terms of the different properties of the exposed material.

Publications

[1] Oberstedt S. and Vanmarcke H., *Recent Investigations on Indoor Radon at SCK-CEN (Mol)*, Euregional Symposium ALMA program of the universities of Aachen, Liège and Maastricht 4. - 6. Nov. 1993, Liège (Abstract accepted by the program committee).

[2] Cornelis J., Vanmarcke H., Landsheere C. and Poffijn A., Modeling radon progency absorbed in glass. Accepted for publication in Health Phys., 1993.

Head of project 2: Dr. McLaughlin

II. Objectives for the reporting period

The main objective for the reporting period was to improve on CR-39 alpha track detectors methods of measuring the area specific activity of recoil implanted Polonium-210 in glass in houses. In order to obtain the maximum benefit from placing a detector in a house a subsidiary objective was to see if a detector configuration could be designed which would measure radon gas and radon progeny plateout in addition to Po-210 in glass. This work was primarily targeted at developing and producing practical alpha detectors for use in houses by contract colleagues such as the group at the University of Gent involved in the Ardennes-Eifel epidemiological study.

Objectives for the next reporting period

It is intended to extend field testing of a new combined Polonium-210/radon gas/radon progeny plateout alpha track detector which was developed during the present reporting period. This detector type will be used in houses being currently investigated for radon exposure by a number of research groups. Detector calibrations will take place with the assistance of contract colleagues. Exposures of this detector type will also take place in a steel radon room within the framework of ongoing laboratory work to determine the effect of air flow and other variables on plateout of short lived radon decay products. It is also intended to continue to carry out laboratory work into techniques to produce short-lived radon progeny alpha sources suitable for alpha track detector energy calibration purposes.

III. Progress achieved including publications

The principal achievements during the reporting period have been (i) the design and produciton of a new configuration of alpha track detector for use in houses and (ii) the development of Monte-Carlo models for the determination of the response of openface and closed radon/radon progeny alpha track detectors. The new detector type consists of a 7 cm square perspex frame onto which alpha track detectors are mounted. On each of the opposite sides of this frame a 3.8 cm diameter disc of the alpha track detecting medium CR-39 is mounted. In use this detector is attached to a glass surface in a room such that one of the discs (detecting surface A) is in firm contact with the glass. One face (detecting surface B) of the second disc faces into a small cylindrical machined cavity within the perspex frame and the other face (surface C) of this disc faces into the airspace of the room. This detector configuration when mounted on a window or other glass surface in a room is designed to give quantitative information on the following:

(a) Recoil implanted Polonium-210 activity in the glass (from analysis of tracks on Surface A).

(b) Contemporary Radon-222 activity concentration in the room air as Surface B over the machined cavity forms a diffusion type closed radon gas detector.

(c) Contemporary Polonium-214 equilibrium surface activity in the room by using surface C as an alpha detector and surrogate for the surface of the glass.

The sensitivity of surface B to radon exposure has been determined as 1.66 tracks $cm^{-2}.kBq^{-1}.m^3.hr$ by means of National Physical Laboratory (UK) standard radon gas sources. Calibrations of surface A to Po-210 in glass exposures have commenced with the University of Gent group.

Surface C is an open-face radon and radon progeny detector. It is meant to act as a surrogate for the glass on which the detector frame is mounted. Because of the alpha detection characteristics of CR-39 it will record alpha particles both from airborne activity and from plateout of radon progeny on its surface.

In the work described here the analysis of the alpha tracks on surface C is primarily airmed at obtaining quantitative information on the Polonium-214 plateout activity. This is achieved by image analysis identification of near normal incidence alpha tracks on surface C which are due to alpha particles greater in energy than about 6.5 MeV. This identification is primarily on the basis of track area. An area based energy resolution of about 0.5 MeV is considered to be sufficient for the present purposes. An empirical approach to achieving this is being investigated in which CR-39 alpha autoradiography of laboratory prepared Po-218/Po-214 and Po-214 sources is used to obtain track area distributions for these emitters. Using the total track area distributions from exposure to these sources a series of track area distribution subsets can be obtained for alpha tracks of increasing degrees of roundness (and hence increasing angle of incidence towards the normal). These distributions can be then used as track area distribution templates to assist in the identification of the near normal incidence alpha tracks on surface C arising only from plateout activity of Po-214. The ratio between the Po-214 surface activity so determined to the recoil implanted Po-210 activity in the glass can then be obtained.

This Po-214/Po-210 ratio will be used as an indicator of any significant difference between historical and contemporary behaviour of radon and its shortlived radon progeny in the house. Such information will be of use in the making of retrospective assessments of radon exposure in houses.

Nearly 70 of the prototype of this new detector have been already distributed for use in houses to groups within the collaboration and to some other radon research groups. The results obtained from these will be analysed both to ascertain the performance of the detectors in the field and to determine the type of contribution they can make to the overall objectives of the collaborative project.

Publications

J.McLaughlin, J. Miles, M. Olast, A. Rannou, L. Tommasino and G. Torri "French/Irish/Italian Field Intercomparisons of Passive Alpha Track Radon Detectors" Rad.Prot.Dos. Vol. 45, 53, 1992.

J.P. McLaughlin and B. Fitzgerald. "A New Technique to Measure the Activities of Short-Lived Radon Progeny Deposited on Surfaces", Rad.Prot.Dos. Vol. 45, 115, 1992

P.O'Connor, V. Gallagher, G. Van den Boom, J. Hagendorf, R. Meller, J. Madden, J. Duffy, J. McLaughlin, S. Grimley, I. McAulay and D. Marsh. "Mapping of Rn and He in Soil Gas over a Karstic Limestone-Granite Boundary. Rad.Prot.Dos. Vol. 45, 215, 1992.

Head of project 3: Dr. Falk

II. Objectives for the reporting period

- Autoradiographic exposure of CR-39 detectors to glass panes with known Po-210 surface activity will continue with the purpose to get enough statistical data for a proper error analysis of the measurement technique.
- The limitation of using this technique seems to be the alpha particles emitted from the natural activity in the glass. Methods based on an absorption technique will be examined.
- A combination of CR-39 and Kodak LR-115 detectors will be studied with the purpose to make an estimate of the background from each individual glass measurement.
- Adoptions of the technique for field measurements will be commenced.

III. Progress achieved including publications

The limitation of using autoradiographic alpha-track methods for the measurement of Po-210 implanted in glass surfaces is mainly due to the alpha particles emitted from background activity in the glass and the different activity level found among a set of glass panes. The energy spectrum of the alpha-particles emanating from the background source differs from the Po-210 surface source. This fact can be used to discriminate the background from the signal.

The method, which we presently are using, is based on an absorption technique. A MYLAR foil coated with a thin layer of aluminium and with a total thickness of 1.8 mg/cm^2 is used as absorber and placed between the glass surface and the CR-39 detector. The thickness of the absorber is chosen so that most of the alpha particles emitted from the surface will penetrate the absorber and leave tracks, while alpha particles emitted from activity within the glass will to a lesser extent leave tracks, due to absorption both in glass and in the absorber.

Autoradiographic exposure with several glass panes with known Po-210 surface activity has earlier been performed and analysed. The relation between the surface activity of

Po-210 and the net track density of the CR-39 detector is found to be the same within a standard deviation of about 15% when the backside of the glass is used as background. It is also found that the sensitivity of the CR-39 measurement technique can be satisfactory if the glasses are exposed to normal or higher levels of radon in houses for more than 15 years.

Figure 1 shows the sensitivity for the CR-39 detector with absorber calculated from measurement on glass panes with known surface activity of Po-210. As shown in the figure the background track density shows a substantial variation between different glass panes. The back side of these glass panes is measured to have an insignificant surface activity of Po-210. A large area pulse ionization chamber (C. Samuelsson, Lund University) has been used for the characterisation of the glass panes used in our experiments.

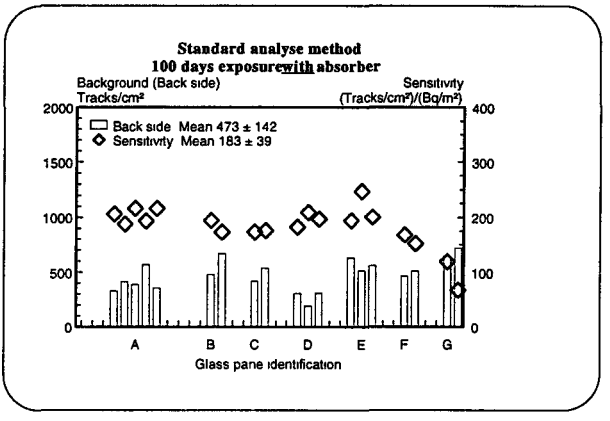

Fig 1. Sensitivity and background track density.

The uses of the track area and shape to obtain an energy distribution of the alpha particles causing the tracks have been tested in small scale. We found that the higher magnification needed during the readout from the microscope caused practical problems. The auto-focusing did not work properly over larger areas and the time to read out and treat the data increased a great deal. After the 3:rd RARE meeting (Lund Dec. 1992) we excluded this approach from our program. Our colleagues in Ireland (McLaughlin, Project 2) will however continue their work on track discrimination techniques.

Two other techniques are under investigation. One is based on two CR-39 detectors with and without absorber and the other uses a combination of two different detectors, CR-39 and LR-115. For both methods it is necessary to ensure that the background alpha activity surface distribution is uniform. The back side of several glass panes were covered with CR-39 detectors and exposed. No significant unevenness could be found. Figure 2 is an example of results obtained from this study.

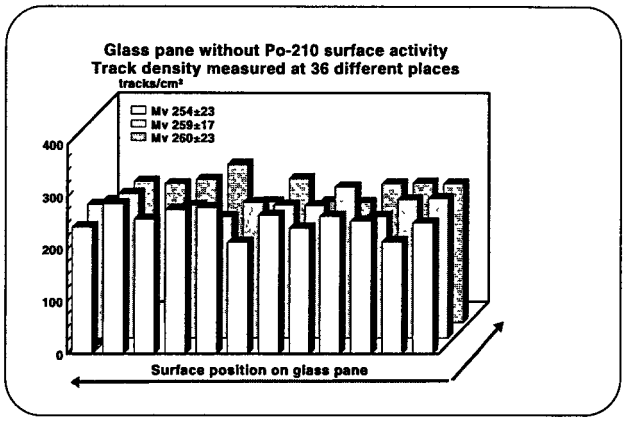

Fig 2. Measured uniformity of background track density.

The reduction of the track density on a CR-39 detector with an absorber is different for the Po-210 alphas compared the background alphas. The vale of the reduction factor for the 1.8 mg/cm² absorber is found to be about 0.8 and 0.5 respectively. This means that results from a combined measurement with and without absorber can be used to estimate the background for that specific glass pane and by that reduce the uncertainty compared to using a mean background value from many different glass panes.

The KODAK LR-115 cellulosa-nitrate film is sensitive to alpha particles with energies in the range 1.2 - 4.8 MeV. This energy range depends slightly on etching and counting conditions. As the alpha particles from Po-210 have an energy of 5.3 MeV they will not be detected unless they are energy degraded. During the reporting period the technique to handle, expose and etch the LR-115 has been set up in our laboratory. Exposures of LR-115 and CR-39 in parallel on glass panes with known Po-210 surface activity is launched.

REFERENCES

H. Mellander and A. Enflo. The alpha track method used in the Swedish radon epidemiological study. Radiation Protection Dosimetry Vol. 45 No1/4 pp. 65-71 (1992).

Head of project 4 : Dr. Poffijn

II. Objectives for the reporting period.

- → Construction of a pulse ionisation chamber as reference for calibration of passive alpha track detectors.
- → Laboratory test of passive alpha track detectors for measuring implanted 210Po activity.
- → First field test with passive alpha track detectors.

III. Progress achieved including publications.

During the last period, a pulse ionisation chamber has been build in Gent. It is an open flow type built out of PVC with aluminium paint covering the outside. The FWHM for ^{210}Po (5.31 MeV) in the present configuration is 116 keV. This instrument will serve as a reference for the calibration of the passive alpha track detectors to be used in field work (see below).

For testing of the ionisation chamber and calibration of the passive detectors, a large alpha source was prepared. This source was prepared by constructing a box of glass with dimension 50x50x2 cm. This glass box was flushed with radon gas in a closed circuit during 105 days . The radon activity was 109 kBq. The spatial distribution of the activity was measured with a surface barrier detector. The average activity (after ingrowth) is 1600 Bq/m^2 with a variation of 15 % over the surface of the glass. This high activity permits us to expose the passive detectors to sufficient alpha radiation in a short period.

Laboratory and field tests were done with passive ^{210}Po detectors. Two type of detectors were used : CR-39 (in collaboration with University College Dublin, Ireland) and Makrofol. The detectors have been designed and calibrated in the lab, by means of the large alpha source. Preliminary tests in houses have been performed with the CR-39 detector. More intense testing in the field will be performed in the near future.

Room Model calculations have been performed on the data obtained during the first tests in houses with the CR-39. Preliminary result of the calculations show that the room model can predict the implanted ^{210}Po activity onto single items within a factor 2-3. More data however is needed to confirm and improve these results.

Publications :

"Modelling radon progeny absorbed in glass", Cornelis J., Van Marcke H. Health Physics 65(4): 414-417.

Head of project 5 : Dr. Samuelsson

II. Objectives for the reporting period

To evaluate an exercise involving new glass-sheets exposed in a high-radon dwelling. To write and test a computer algorithm to facilitate the evaluation of long-lived daughter activity of glass sheets exposed to non-constant radon concentrations. To design a new open flow pulse ionisation chamber of a modular design. To expose clean and unclean household glass panes in the radon steel room during well known conditions in order to see the influnce from dirt and dust on the implantation process.

Objectives for the next reporting period

The objectives for the next reporting period are to investigate the high-radon house more in detail. The exposure of initially unexposed objects will continue and the Po-210 measurements will be extended also to existing glass objects in the house. Long-term measurement of wall temperatures close to the exposed objects will be performed. Both pulse ionisation chambers and autoradiographic track-etch devices will be intercalibrated among the RARE contractors and external laboratories by means of reference large-area glass samples. Methods suitable for long-term integration in homes of ultrafine radon daughters and aerosol-bound activity will be evaluated theoretically and by experiments in a radon room.

III. Progress achieved including publications

Po-210 implanted into four new glass sheets placed at three different rooms in a high-level radon house has been followed since December 1990. The house has been inhabited by the same family for more than 40 years and today the family consists of an elderly couple. The radon infiltrates the house from the ground.The objective of this exercise, still in progress, is to see to what extent the implanted Pb-210 depends on position and how well radon exposure and implanted Pb-210 correlates. The glass sheets covering framed photographs have been the same throughout the exposure and are attached with two radon track-etch cups each. The radon cups are exchanged and evaluated on a regular basis by SSI. The implanted ^{210}Po into the glass sheet is determined by alpha spectrometry in our laboratory each time a new set of radon cups is inaugurated. Concerning exposure geometries, the frame denoted B leans towards the wall, close to the ceiling, above a bookshelf in the living-room, frame S hangs in a corner of the dining area with a close staircase just above, and the two frames W1 and W2 hang on the wall one above the other in the bedroom. None of the frames is placed close to ventilation openings or radiators. The radon results are displayed in Figure 1.

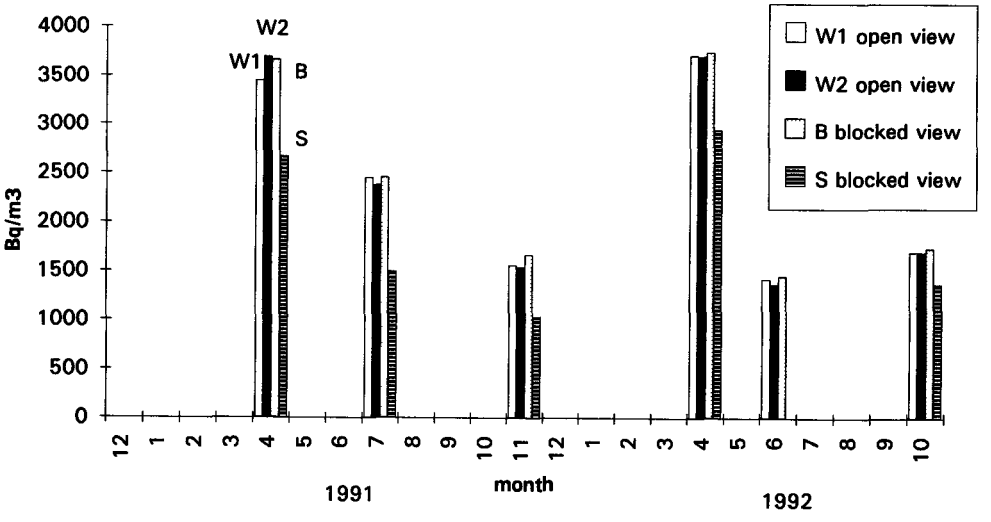

FIGURE 1. *The radon concentration at each glass-sheet position. The radon values refer to the time interval prior to each measurement.*

During evaluation it is assumed that the radon levels have been constant within each measuring period. In order to handle ingrow and decay corrections a computer algorithm has been written. By the algorithm each experimental Po-210 result is converted to Pb-210 activity, taking ingrowth of Po-210 from earlier exposure time intervals into account. The implanted Pb-210 of the four glass sheets is shown as a function of radon exposure in Figure 2.

The results in Figure 2 support the opinion that only glass-sheets exposed in similar geometries give comparable results. The radon concentration in the dining-area (frame S) is apparently lower than in the two other rooms. There is a tendency of concave curvature in Figure 2, especially for glass-sheet S in the dining area. This deviation from a linear increase may be due to the change in smoking habits that took place in the family during 1991.

Aiming at an improved energy resolution and a more flexible design a modified PIC detector is now under construction with help from the workshop at DTH, Denmark (Niels Jonassen).

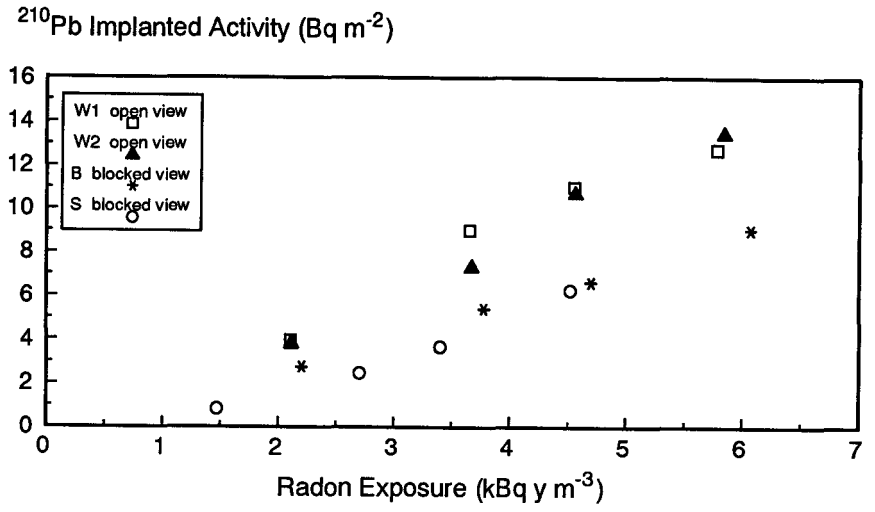

FIGURE 2. *The Pb-210 activity, as calculated from Po-210 measurements, of four glass-sheets exposed to radon in three different rooms in a radon dwelling. The implanted activity of the frames exposed in recessed places (B and S) is approximately 40% lower than that in an open geometry (W1 and W2).*

The influence of dust on the adsorbed and alpha recoil implanted Po-210 activity has been studied in radon room experiments. Awaiting the ingrowth of Po-210, only a limited number of objects have been analysed and it is too early to draw definite and quantitative conclusions at this stage. However, one observation concerning the experiments is clear. The removable fraction (by cleaning after radon exposure) of Po-210 varies more than expected both for initially clean and initially dirty glass-sheets. This variation is presumely due to the fact that loosly adsorbed Po-210 can be lost during exposure, handling, and measurement. The eventual disappearance of adsorbed Po-210 does not interfere with the ability of using Po-210 as a retrospective monitor for radon as, on glass surfaces, only the part of Po-210 (and Pb-210) that is protected from removal by alpha recoil implantation is useful for this purpose.

Publication

Samuelsson C. and Johansson L., *Long-Lived Radon Decay Products as a Long-Term Radon Exposure Indicator.* CEC. First International Workshop on Indoor Radon Remedial Action. Rimini-Italy 27 June-2 July, 1993.

Progress Report

Contract: **FI3P-CT920034** **Sector: C12**

Title: Characteristics of airborne radon and thoron decay products.

1)	Porstendörfer	Univ. Göttingen
2)	Poffijn	Univ. Gent
3)	Vanmarcke	CEN/SCK Brussels
4)	Akselsson	Univ. Lund
5)	Falk	SSI - Stockholm
6)	Tymen	Univ. Brest
7)	Ortega	Univ. Catalunya - Politécnica

I. Summary of Project Global Objectives and Achievements

Besides the airborne activity concentrations, the size characteristics of the unattached and attached decay products and the quantity of unattached activity are important parameters in all models for dose calculations. The information on the size characteristics are also essential for understanding and describing the behaviour of the airborne activity in indoor and outdoor environments. For example, the retrospective assessment of indoor exposure to radon (^{210}Pb in smooth surfaces) needs information of the variation and size dependence of the airborne activities.

Values for the size distribution of the unattached and aerosol-attached activity in the domestic environment and the magnitude of the unattached activity are quite limited and consequently uncertain. The information available in the literature shows that it is necessary to determine aerosol and room conditions (such as aerosol particle concentrations, ventilation, type of aerosol and vapour sources, humidity) during measurements. For the correct determination of the real variation of these parameters under normal living conditions more efforts have to be done to reduce uncertainties concerning activity measurements, to calibrate and improve size fractionating instruments, to compare different data evaluation methods, and to simulate the behaviour of airborne activities by model calculations.

The points of emphasis of the current project are to determine in the domestic environment the real variation of F, f_p, and the variation of the complete activity-weighted size distribution. To estimate the influence of different living conditions of the habitants in various geographical and climatic regions of Europe and to study the influence of different kinds of aerosol sources and sinks (cigarette smoke, air cleaning) on these parameters are important aspects. These measurements in realistic, domestic environments are completed by controlled chamber studies considering the physical and chemical interaction (particle growth, cluster formation, plate-out rates) of unattached radon and thoron decay products with trace gases (e.g. humidity, SO_2) and other aerosol particles.

Besides the determination of these input parameters for dose calculations, other methods for dose determinations are developed and improved: simulation of lung-deposited activities by a combination of diffusion screens and direct measurements of lung-deposited activities in a whole-body-counter.

For size analysis of the unattached and aerosol-attached activities in the complete size range of ions and clusters (~ 1 nm) up to larger aerosol particles (several µm) different experimental techniques are used by all groups involved in this project: diffusions techniques, electrostatic classification methods, and various kinds of impactors. Inactive number size distributions will mostly be measured by electrostatic classification in connection with condensation nuclei counters and in the larger size range by light scattering methods.

A method for long-term measurements of the ultrafine decay products using an alpha track detector (LR 115) was developed and tested.

Although a separate agreement with the non-CEC-member Sweden was not signed during the reporting period, the aerosol groups of Lund university and SSI Stockholm continued their co-operation in this radiation protection project.

Head of project 1: Porstendörfer

II. Objectives for the reporting period

a) reporting period

- Test and calibration of size fractionating units (classifier, single screens) for radon chamber studies.
- To set up and test an electrostatic classifier (TSI, Model 3071) in connection with a condensation nuclei counter (TSI, Model 3025) and an unit for alpha-spectroscopy.
- Final data evaluation and conclusions of intercomparison measurements concerning different size fractionating methods.

b) plans for next reporting period

- Test and calibration of size fractionating units (classifier, single screens) for radon chamber studies will be continued: e.g. improvement of size resolution of classifier, minimisation of particle losses, determination of back/front ratio of single-screens.
- Size distribution measurements of inactive and aerosol-associated activities with the electrostatic classifier are planned in a room with elevated radon gas concentrations.
- Size distribution measurements with an open-face screen diffusion technique (0.5-20 nm) will be continued in a normal living room at different aerosol conditions

III. Progress achieved including publications

1) METHODOLOGY

a) The major parts of a radon chamber (0.05 m^3) - for studying the influence of trace gases and humidity on cluster formation processes in the diameter range 0.5 - 20 nm - are an electrostatic classifier and a rotating screen diffusion disk. These units were built in the machine shop of the IL. For calibration and for checking of the performance of these devices an aerosol generator was re-activated for producing monodisperse aerosol particles (NaCl, Ag) in the diameter range of 3 - 200 nm. First test measurements yield that some parts of the inlet system of the classifier had to be modified.

b) For the measurement of activity - weighted size distributions in the nucleus mode size range with diameters between 10 - 100 nm experimental methods based on the analysis of the electric mobility of aerosol particles have the best size resolution, compared to impactors and diffusion techniques. To improve the sensitivity for this technique for radioactive aerosol particles and for modelling radioactive and inactive size distributions, an electrostatic classifier in connection with a condensation nuclei counter (CNC) and a unit for alpha-spectroscopy was built up and tested. For this device a computer program was written and hardware modifications were done to perform automatic and continuous measurements. From the particles losses inside the classifier, the charging probability of the aerosol particles and the response function of the CNC it can be estimated that the detection limit for size analysis of number size distributions is about 4-5 nm and 250 nm, respectively.

The activity of RaA - attached particles can be measured continuously after electrostatic classification by alpha spectroscopy using a surface barrier detector. First test measurements in a small radon chamber yield that sufficient counting statistics also for particle sizes of about (10 - 20) nm can be achieved in atmospheres with elevated radon gas concentrations of 2000 - 3000 Bq/m^3 corresponding to concentrations of RaA of 1000 - 2000 Bq/m^3.

Parallel to the method of electrostatic classification an optical system (LAS X) for size analysis in the diameter range between 200 - 7000 nm was tested and a computer program was written to support automatic measurements.

2) RESULTS

Side-by-side measurements were performed in Northern Bavaria (Germany) in a house with elevated radon gas concentration by the following participants: EML of the US-DOE, New York, USA; ARL of the Department of Community and Health, Yallambie, Australia and the IL of the University, Göttingen, Germany.

One important objective of this intercomparison was to clarify if the methods for size analysis of these three laboratories yield equivalent results with regard to finally derived dose conversion factors.

Although the results of the determined activity size distributions of the single measurements are sometimes considerable different the averaged size distributions (three days) are in agreement on the main points. In table 1 the averaged size distributions of ^{218}Po in the diameter range < 10 nm are compared. Although the median diameters of this size distributions agree only within a factor of two (d_1 = 0.54-1.2 nm; D= 0.036-0.17 cm^2/sec), the influence of these differences on the calculated average effective dose can be neglected (difference < 30%). However, the difference of total amount of the "unattached" ^{218}Po (34-54%) is significant. The data evaluation sometimes yielded bimodal ^{218}Po distributions, with the additional mode in the size range 4-5 nm. This finding is important for future studies to understand the behaviour of radioactive aerosols or clusters in the environment; but the current dose models [1,2] show only minor influence on the calculated dose conversion factors using unimodal or bimodal distributions of the "unattached" activities.

In table 2 the results of the complete size distribution of the PAEC, measured by different screen diffusion techniques of ARL and EML are summarised. The weighted effective HE-DCFs of the daily-averaged measurement differ less than 30%. The sizes of the aerosol-attached activities of the accumulation mode (AMD = 192-297) are quite different.

Activity size distributions of ^{214}Pb performed with different kind of low-pressure impactors of EML and IL are summarised in table 3. The difference of the median diameters of the accumulation mode (AMAD=238-332) is surprising. However, both methods clearly show that a significant amount of ^{214}Pb is associated with aerosol particles in the diameter range 10-100 nm and sometimes in the diameter range of the coarse mode (~2000 nm). This findings are very important for correct dose calculations. The data of table 3 show the problem of accurate particle size measurements in the diameter range 10-100 nm.

From the results of these intercomparison can be concluded that there is agreement on the main points, but some disagreements on details of peak location and shape. However, most of this disagreements do not affect dose calculations very much. But these joint measurement clearly showed that accurate measurement of activity size distribution in the diameter range 10-100 nm, especially the range below the cut-size of the last impactor stage, is a difficult task that has not been fully solved as yet. The amount of short-lived radon decay products in this nuclei size range will have a major influence on the calculated dose. Furthermore this study show that it is necessary to have a closer look to the coarse mode size range with diameters of some microns. Additional measurements are necessary to clarify if the measured activity in this size range is real or if it is an artefact of the method or a misinterpretation of smaller particles.

References

1. James, A.C.. Gehr, P., Masse, R., Cuddihy, R.G. Cross,F.T., Birchall, J.S. Durham, J.S. and Briant, J.K. *Dosimetry model for bronchial and extrathoracic tissues of the respiratory tract.* Radiation Protect. Dosim. 37, 221-230(1991).
2. James, A.C. Private Communication, May 1993

Table 1: Activity size distributions of the short-lived radon decay product ^{218}Po in the diameter size range 0.5-10 nm: average results of measurements performed during three days in a moderate ventilated room ($v < 0.5\ h^{-1}$) at average aerosol particle concentrations of $Z = 4450\ cm^{-3}$ (range: 2400-8000 cm^{-3}). The corresponding weighted effective dose conversion factors HE-DCF(mSv/WLM) were derived from ICRP Task Group Model LUDEP, Version May 1993 (1,2).

Group/no. of measur.	method	d_1(nm)	σ_1	f_1 (%)	d_2(nm)	σ_2	f_2 (%)	total weighted HE- DCF (mSv/WLM)
IL Göttingen 7	Simplex unimodal	1.05	1.62	54				34.4
	EM unimodal	0.84	1.76	52				30.6
	Simplex bimodal	0.84	1.01	41	3.16	2.59	17	34.5
	EM bimodal	0.77	1.16	42	3.88	1.42	10	29.5
ARL Yallambie 8	EM	0.59	1.15	20	2.80	1.20	20	27.3
	Twomey	0.54	1.11	19	2.09	1.40	43	45.5
EML New York 5	EM	1.19	1.20	34				22.3

Table 2: PAEC size distributions measured with screen diffusion techniques from EML and from ARL.

		"unattached" activities			accumulation mode		
Date	group	AMD (nm)	σ_g	fraction (%)	AMD (nm)	σ_g	fraction (%)
2 Oct. 1991	EML	1.2	1.4	18	173	2.5	82
	ARL	0.85	1.2	13	303	2.6	87
3 Oct. 1991	EML	1.25	1.3	11	222	2.0	89
	ARL	1.12	2.0	15	248	2.3	85
4 Oct. 1991	EML	1.1	1.3	14	180	2.2	86
	ARL	0.59 4.5	1.4 1.5	18 15	333	2.5	67

Table 3: Size distributions of aerosol-attached ^{214}Pb activities measured with a low-pressure cascade impactor (BERNER) and screens in series (IL, Göttingen) and a low-pressure MOUDI impactor (EML, New York).

		Nuclei mode			Accumulation mode			Coarse Mode		
Date	group	AMAD (nm)	σ_g	fraction (%)	AMAD (nm)	σ_g	fraction (%)	AMAD (nm)	σ_g	fraction (%)
2 Oct. 1991	IL	27	2.1	32	367	1.6	44	~2000	?	24
	EML	< 50	?	18	235	1.9	70	1690	1.4	13
3 Oct. 1991	IL	51	< 1.2	25	353	2.1	74	~2000	?	1
	EML	< 50	?	19	248	1.9	75	1790	1.4	6
4 Oct. 1991	IL	48	< 1.2	26	255	2.3	66	~2000	?	8
	EML	< 50	?	35	225	2.0	61	1830	1.4	4
2-4 Oct 1991 average	**IL**	**44**	**< 1.2**	**27**	**332**	**2.0**	**65**	**~2000**	**?**	**9**
	EML	**< 50**	**?**	**22**	**238**	**1.9**	**70**	**1760**	**1.4**	**8**

3) PUBLICATIONS

A. Reineking, G. Butterweck, J. Porstendörfer, J.C. Strong, H. Vanmarcke, R. Van Dingenen
"Intercomparison of methods for investigating the physical characteristics of radon decay products in the indoor environment", Radiat. Prot. Dosim. 45, 41-46 (1992)

G. Butterweck, J. Porstendörfer, A. Reineking, J. Kesten
"Unattached fraction and the aerosol size distribution of the radon progeny in a natural cave and mine atmospheres", Radiat. Prot. Dosim. 45, 167-170 (1992).

J. Porstendörfer, A. Reineking
"Indoor behaviour and characteristics of radon progeny", Radiat. Prot. Dosim. 45, 303-311 (1992)

A. Reineking, G. Butterweck, J. Kesten, J. Porstendörfer
"Thoron gas concentration and aerosol characteristics of thoron decay products", Radiat. Prot. Dosim. 45, 353-356 (1992)

A. Reineking, G. Butterweck, J. Kesten, O. Malolepsy, B. Kopka, H.-J. Heymel, E. Speer, J. Porstendörfer
"A monitor for continuos and simultaneous measurement of environmental thoron and radon gas" Proceedings of thoron/thoron progeny intercomparison, (Editor J. Bigu), Elliot Lake, Canada, November 2-6, 1992

P.K. Hopke, R. Strydom, M. Ramamurthi, E.O. Knutson, K.W. Tu, P. Scofield, R.F. Holub, Y.S. Cheng, Y.F. Su, W. Winkelmayr, J.C. Strong, S. Solomon, A. Reineking
"The Measurements of Activity-Weighted Size Distributions of Radon Progeny: Methods and Laboratory Intercomparison Studies", Health Physics 63, 560 - 570, 1992

J. Kesten, G. Butterweck, J. Porstendörfer, A. Reineking, H.-J. Heymel
"An Online α-impactor for Short-Lived Radon Daughters", Aerosol Science and Technology 18, 156-164, 1993

A. Reineking, J. Kesten, G. Butterweck, J. Porstendörfer, E.A. Knutson, A.C. George, S.B. Solomon
"Size distributions of unattached and aerosol-attached short-lived radon decay products:some results of intercomparison measurements", first international workshop on indoor radon remdial action, Rimini, Italy, June 27 - July 2, 1993

Head of project 2 : Dr. Poffijn

II. Objectives for the reporting period.

→ Measurements of aerosol distributions in houses.

III. Progress achieved including publications.

During the last period the aerosol equipment of our group has participated in an inter-comparison exercise done by Rita Van Dingenen at ISPRA (Italy)[1]. This exercise consisted of two parts : lab tests under controlled conditions were the accuracy of the equipment in the different measuring modes was tested and field measurements during the "Hudson Cruise", a transatlantic measuring campaign to collect data on general pollution of the air. The results of this exercise are :

→ The Condensation Nucleus Counter (CNC) did not operate well in the photometric mode due to a malfunction in the cooling section. This malfunction has now been repaired.

→ The number of particles in the lower channels is overestimated due to a difference between the adjusted voltage and the measured voltage on the central rod. This can be corrected by changing the control software for the EC-CNC equipment. This is only necessary for aerosol distributions completely under 0.1 μm.

After this comparison exercise, the equipment returned to Gent.

Detailed measurements of the indoor air conditions were performed in two houses with known radon problems. Along with the aerosol information, radon and radon daughter concentrations were measured. Simultaneous measurements were performed by the "SCK" (Mol, Belgium) group with the "Bronchial Dosimeter". Analysis of this data in progress.

In the future, more detailed measurements will be done in houses with known radon concentrations.

[1] Unpublished reports by and personnel talks with Rita Van Dingenen

Head of project 3 : Dr. Vanmarcke

II. Objectives for the reporting period

Investigations on possible differences in plate-out rates between the different unattached short-lived radon daughters.

Measurements with the BRONCHIAL DOSEMETER are planned in several dwellings in the Ardennes region. The radon concentration and the bronchial and nasal deposition of its decay products will be measured. In collaboration with the university Ghent (project 2) the aerosol concentrations and the inactive size distribution will be measured in order to evaluate the equilibrium factor and the activity median diameter of the attached fraction.

III. Progress achieved including publications

In collaboration with the university of Ghent a measurement campaign started with dwellings in Visé and Cheratte both situated in the Ardennes region around Liège. Due to extreme windy and hot weather the radon concentration in the Visé-dwelling dropped below 100 Bq/m^3. Also in the Cheratte-dwelling only 400 Bq/m^3 was found. Under these conditions particularly the electronic part of the dosemeter as well as the multi-channel analyser did not work properly. The high temperature led to high instabilities and gain shifts which could not be corrected on spot. Therefore we decided to interrupt this campaign and to setup a new BRONCHIAL DOSEMETER with the following improvements :

1. the pre- amplifiers and amplifiers of each α-particle detector have been replaced by individual high quality modules;

2. the vacuum tubing has been re-installed serving for faster and more precise handling of the pressure inside the α-particle detectors;

3. the multi-channel analyser was replaced by a PC-AT compatible system consisting of a MCA-memory card together with a complete MCA-emulation PC-software (SILENA).

The mechanical part of the re-installation is now completed and the setup of the electronical part is just under way. The re-calibration of the BRONCHIAL DOSEMETER will be done in october '93 followed by a laboratory test-period of about one month. A new measurement campaign is planned during the winter period 93/94.

Publications

[1] Oberstedt S. and Vanmarcke H., *Recent Investigations on Indoor Radon at SCK-CEN (Mol)*, Euregional Symposium ALMA program of the universities of Aachen, Liège and Maastricht 4. - 6. Nov. 1993, Liège (Abstract accepted by the program committee).

Head of project 4 : Professor Dr. Akselsson

Objectives for the reporting period

The objectives for the reporting period were to further develop a multi-orifice impactor for radon progeny size measurements, to investigate the possibility of measuring the hygroscopicity of indoor aerosols and to investigate radon progeny behaviour in a simulated dwelling.

Progress achieved including publications

During the reporting period the multi-orifice (orifice diameter=50 mm) impactor has been optimized with respect to orifice density to get sharp cut-offs for particle diameters below 100 nm (figure 1). Work is in progress to maximise the size off the nozzle plate, and thereby the air flow, while maintaining good cut-off characteristics.

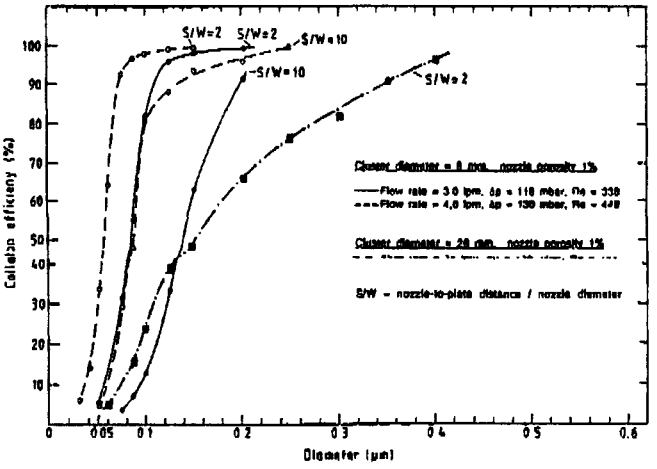

Figure 1. Collection characteristics of the multi-orifice impactor for different Reynolds numbers and S/W ratios (S=orifice-to-plate distance, W=orifice diameter)

Within the aerosol group of Lund University a system for measuring hygroscopic growth of particles has been developed. The system consists mainly of a so called Tandem Differential Mobility Analyser (TDMA). The TDMA (figure 2) separates the aerosol into narrow size fractions and measures the diameter of the particles before and after humidification. At this moment the highest possible humidity in the humidifier is 85-90 %, and this is to low to mimic the respiration tract of a human being. We hope to be able to increase this to 95-99 % and thereby use this system to measure the hygroscopicity of typical indoor aerosols. This knowledge is important for making more accurate dose calculations.

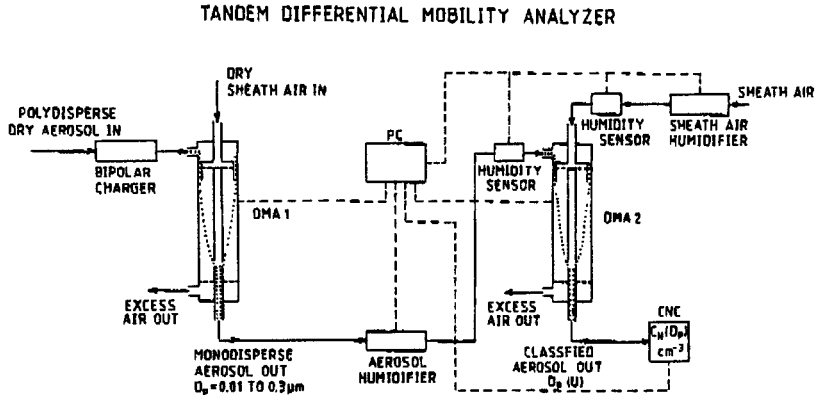

Figure 2. Block diagram of the TDMA setup.(From: Svenningsson et al. "Hygroscopic growth of aerosol particles in the Po Valley", Tellus, 44B, pp 556-569 (1992).

In the radon exposure chamber at our institute a study was performed to investigate the unattached fraction of radon progeny close to, and far away from the human face, respectively. The results showed no difference between the two situations (ref. #1, # 2). A study that has started recently deals with turbulent deposition of particles onto the surfaces of a room. This is especially important for the retrospective radon monitioring (CEC project FI3P-C1920025), since large variations in deposition velocity is often seen in those studies. Our objective is to establish how large these variations in deposition velocity are in dwellings, due to different aeresol concentrations, ventilation, heating etc. At this point there are no conclusive results from this study.

Objectives for the next period

The objectives for the next period is to further develop the multi-orifice impactor for use as a radon progeny size analyser This means maximising the flow to increase sensitivity. We also plan to develop the TDMA system so that it can be used to simulate growth in the human airways. The study of turbulent deposition will continue.

Publications

#1. "The Effects of Thermal Convection Flow on Aerosol Properties and Unattached Fraction of Radon Daughters Close to the Human Face." Proceedings of the Symposium of the Nordic Society for Aerosol Reasearch, Solna, Sweden, 1992.
#2. "Aerosol Properties and Unattached Fraction of Radon Daughters Close to the Human Face." Proceedings of the First International Workshop on Indoor Radon Remedial Action, Rimini, Italy, 1993.

Head of project 5: Dr. Falk

II. Objectives for the reporting period

- Modification of the experimental technique for the continuation of deposition studies on radon progeny in humans.
- Size determination of the unattached fraction of the three radon daughters using wire screen technique.
- Measurements of ^{220}Rn and ^{220}Rn progeny indoors in some Swedish dwellings and if possible determine the ^{220}Rn progeny unattached fraction.

III. Progress achieved including publications

The experimental study to determine the fraction of inhaled radon daughters deposited in the human air ways has been carried out with a combination of two different techniques. The total amount of radon daughter deposited during the exposure was determined by measurement of the radon daughter concentration in inhaled and exhaled air. The sites of deposited radon daughters were immediately after the end of the exposure assessed by external γ-measurements of the subject in a low level whole-body laboratory.

The experiments carried out during previous reported period was focused on the different deposition pattern between the "unattached" fraction and the radon daughters carried by an aged aerosol. The experiences acquire from these studies show difficulties to have control of necessary parameters when the exposure was performed with high unattached fraction. Plate-out on clothes and body surfaces added to the difficulties.

A new design of the arrangements for the collection of exhaled radon progeny has been developed and will be used in the continuation of the study. In principle the exhaled air is sucked through a filter placed at very short distance from the exhalation mouthpiece. In order obtain a breathing condition as natural as possible a pressure gauge attached to the mouthpiece controls the speed of the air-pump sucking the exhaled air through the filter. By these arrangements no breathing force is needed independent of breathing rate. See fig 1.

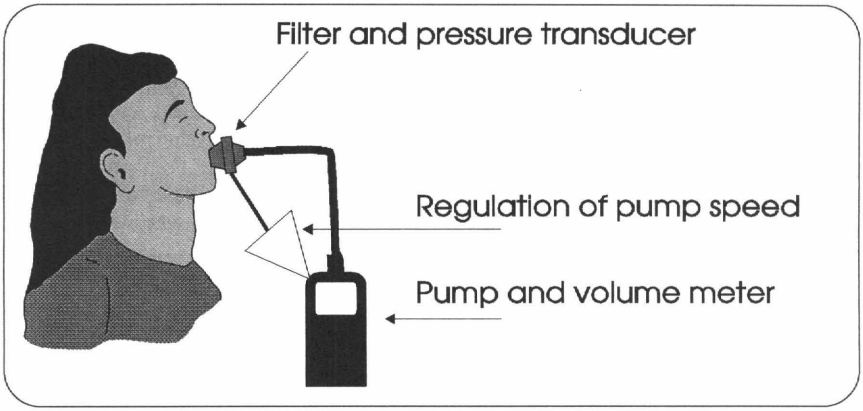

Fig 1. New design of apparatus for collection of exhaled radon progeny.

The determination of the size for the unattached fraction of airborne radon progeny has been performed in a test-chamber inside a walk-in radon-room. The aerosol concentration in the room was lowered to less than 1000 particles/cm3. With an additional filter for the test-chamber the aerosol concentration during the study was typical less than 200p/cm3 giving practically no attached radon progeny in the test atmosphere.

Using different flow-rates and different mesh sizes for the wire screens, the collection efficiency of the unattached daughters could experimentally be determined. Knowing the characteristics dimensions of the screen used and the flow-rate, the aerodynamic size of the unattached fraction could be calculated by use of the screen-type diffusion battery theory. A significant difference in size of the unattached Po218 and Bi214 was found. As can bee seen in fig 2 the results are in agreement for the three different mesh sizes used. The results is valid only for clean indoor laboratory air.

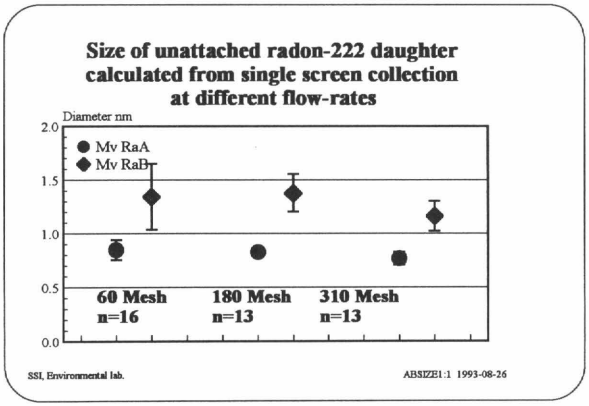

Fig 2. Measured size of unattached radon progeny

Measurement of ^{220}Rn progeny has been undertaken in 61 dwellings at 13 different places. The dwellings were not random selected, but could be expected to show higher ^{220}Rn progeny levels. The measurements were performed at two locations in each dwelling, living-room or bedroom and basement lounge or other room in the basement. Typical values of EET in dwelling was found in the range 0.1 to 0.4 Bq/m³ with maximum value of 16 Bq/m³. The unattached fraction was measured to be in the range 0.003-0.03 Bq/m³ with a maximum value of 0.8 Bq/m³. The EET values found from measurements in the basements are typically 0.3 - 1.5 Bq/m³ with a maximum value of 12 Bq/m³. The unattached fraction was measured to be in the range 0.01 -0.05 Bq/m³ with a maximum value of 6.3 Bq/m³. A few measurements of the ^{220}Rn gas were performed in the basement and showed typically values between 10 and 80 Bq/m³ with a maximum value of 430 Bq/m³. These reported results are preliminary as the analysis of the collected data still is in progress.

Measurement of ^{220}Rn has been performed in a part of a random selected group of dwellings in Sweden. The preliminary results from 31 dwellings show a mean-value of 13 Bq/m³, a median of 4 Bq/m³. A single high value of 150 Bq/m³ was also found. All the ^{220}Rn results reported here are one day mean-values.

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R Falk, H Möre and L Nyblom . Measurements of ^{220}Rn in air using a flow-through Lucas cell and multiple time analysis of recorded pulse envents. Radiat. Prot Dosimetry. Vol 45, No 1/4, 111-113, 1992.

Head of project 6: Dr. Tymen

II.Objectives of the reporting report.

One of the main ojective of this second part of the contract was to improve the α-activity counting procedure of radon daughters collected by the SDI 2000 sampling device in indoor environments. Finally, the choice was given to the carrying-out of a nine-channel counting system including nine PIPS dectectors, five of 450 mm^2 area and four of 2000 mm^2 area, assigned to count α-activity on respectively the filters and the 4 last plates of the Andersen impactor constituting the SDI 2000. The global counting protocole was planned to be conducted form a PC computer on the basis of a deconvolution procedure perfected at the Commissariat à l'Enrgie Atomique. Otherwise, it was necessary to modify the geometry of filter-holders downstream the granular beds of the SDI, in order to get out them as fast as possible before the counting procedure.

Parallely, a second program was initiated aiming to measure unattached fraction of Rn-daughters, time integrated over a large sampling time, based on the use of an alpha track detector as support of free radon daughters collected in an appropriated sampling system. The sampler consist in two coaxial cylinders forming an annular diffusion channel of 30 cm lenght, 2;5 mm width (in the last configuration) designed to received a LR115 Kodak film wound on all its surface, itself covered by a 13μm mylar foil in order to register ultrafine Po^{218} only.

This research was made in collaboration with the "Laboratoire de Physique et Métrologie des Aérosols" (LPMA) of the Commissariat à l'energie Atomique (Dr. BOULAUD).

III. Progress achieved including publications.

Concerning the first program, in spite of several technical problems in the building of the counting apparatus which caused an unforeseen delay, the prototype is now pratically ready for the first tests. Based on the Time Evolved Least Square method, the activity data teatment will allow a better determination of individual RnD size distribution. New field experiments will be intensively performed just after the preliminary tests that is to say inside the three next months.

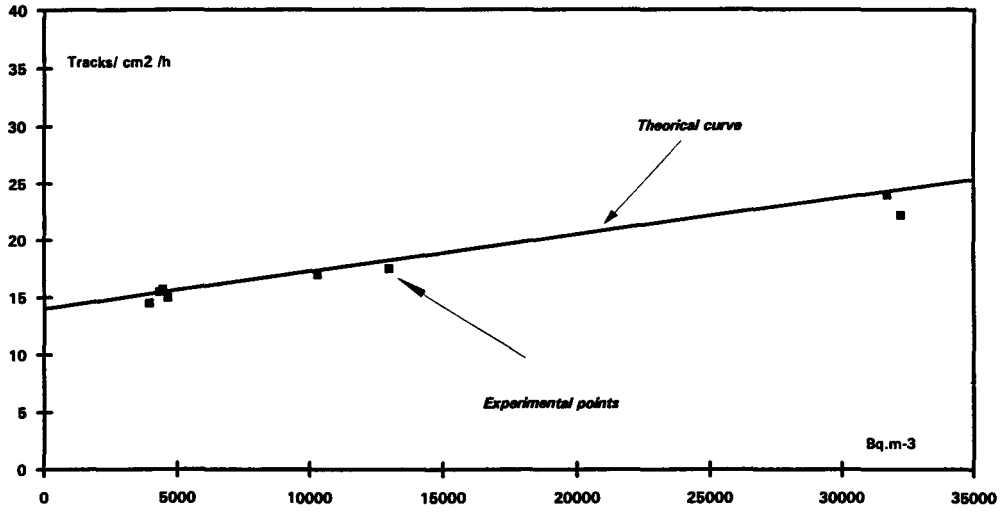
In the frame of program n°2, a lot of preliminar experiments were conducted in a radon chamber of 0.3m^3 in order to examine the behavior of the annular diffusion channel for the time intagrated of the unattached component. Different experimental conditions were possible by introducing in the chamber either radon gas alone or radon with particles. During the experiments, the unattached fraction was measured by a classical screen method. After the sampling, the film detector was etched for 140mn in 2.5N analytical grade NaOH at 60°C with on stirring, in a thermo controlled water bath. After the etching and a washing of the film, the number of alpha tracks was automatically counted on little stips throught a sparking counting technique. First results gave more tracks due to unattached Po^{218} than expected from a theoretical estimation. In fact it was taken in evidence the direct influence of the radon gas in the production of tracks . To quantify this phenomenon another series of experiments were performed where the inlet of the annular diffusion channel was closed by an absolute filter so that only radon gas was able to make tracks in the inter cylindrical

space. Fig. 1 shows a fairly good agreement between the number of alpha tracks effectively counted and the estimated number.

So the prototype can be considered now as ready and well caracterized for calibration in different conditions. According our estimations and taking into account the various correction factors involved, the influence of radon gas on the track density, should be of about 10% over a month exposure in a 200 Bq m^{-3} radon concentration and assuming 20% of the Po^{218} to be in an unattached form. Otherwise, the study of the track density all along the lenght of the nitrate film should give informations on the size of collected radon daugthers.

In parallel this experimental work, we have also studied the penetration performances for unattached fraction throught the classical theory of diffusion but applied to an annular space of internal and external radius R_1 and R_2. By solving the general equation of the diffusion in steady state conditions, thanks to the Sturm-Liouville theory, we were able to obtain an analytical solution of the efficiency of the annular channel depending only of the ratio R_1/R_2. It was also noted that the efficiency curve of an annular channel is all the more comparable to this of a rectangular channel because R1/R2 is near to 1.

Fig. 1. Influence of Rn-222 on track density in the annular diffusion channel



Publications.

1 - **M.C. ROBE, A. RANNOU, G. TYMEN, J. LE BRONEC.**
"Radon diagnosis based on investigation of radon sources and radon entry in houses".
Fifth International Symposium on the natural radiation environment. Salzburg, Austria. 22-28 septembre 1991,
Radiation Protection Dosimetry, Vol. 45, n° 1-4, 1993, pp. 319-322.

2 - **G. TYMEN, M.C. ROBE, A. RANNOU.**
"Measurements of Aerosol and Radon Daughter size distributions in five radon houses".
Fifth International Symposium on the natural radiation environment. Salzburg, Austria. 22-28 septembre 1991,
Radiation Protection Dosimetry, Vol. 45, n° 1-4, 1993, pp. 391-393.

3.- **A.M.GOURONNEC, M.C.ROBE, N.MONTASSIER, D.BOULAUD, G.TYMEN, A.RENOUX**

"Modelling of the behavior of radon and its decay productsin dwellings andexperimental validation of the model". *Indoor air congress 1993, Helsinski*. To be published in *Indoor Air.*

4- In preparation :" Long time measurement of unattached fraction of Rn^{222} daugthers by means of an annular diffusion channel with a track detector film" by **D.KEROUANTON, G.TYMEN, D. BOULAUD.** to be presented for publication in *J.of Aerosol Science.*

Head of project 7: Dr. Ortega

II. Objectives for the reporting period

- To prepare a young researcher in the aerosol studies.
- To set up an automatic facility, based on german groupe technique, which permits to measure radon and thoron and radon daughters concentration.
- To study the error sensibility of different parameters in the measurements.

III. Progress achieved including publications

- It has been carried on a preliminary survey campaign over 15 dweeling in the city of Barcelona. It has been used two passive techniques: charcoal detectors and track technique. This campaign will be repeated in the next seasons.
- It has been implemented an automatic facility based on Göttingen Isotopenlaboratorium group techniques with the aim of doing measurements over some specific sites in order to know the parameter radon concentration, radon daughters concentration in air and attached and unattached fraction.
- An error calculating procedure has been performed with the aim of knowing the sensitivity of different parameters of the detection system. This knowledge will permit us to improve it.
- Stay of a week of a young researcher in Gottingen which participated in a radon electrostatic detector calibration.

PLANNED WORK FOR THE PERIOD (1.6.1994/30.6.1995)

1 - Setting up of a Radon chamber used for checking the detection systems and their improvements, and the influence of different aerosols.

2 - Improvement of the experimental facility for measuring Radon and Thoron concentrations, Radon daughters concentration and attached and unattached fraction. Influence of the humidity.

3 - Measurements of aerosol size distribution and their activity by means of a particle counter (TSI-3025) and screen and diffusion batteries techniques.

4 - Measurements of radon and thoron daughters aerosols into a number of selected dwellings in Barcelona area.

Progress Report

Contract: **FI3P-CT920061** **Sector: C12**

Title: Study of the different techniques to mitigate high radon concentration levels disclosed in dwellings.

1)	Sabroux	CEA-IPSN
2)	Torri	ENEA-DISP
3)	Ortins de Bettencourt	DGA-DPSR
4)	Quindós Poncela	Univ. Cantabria
5)	Kritidis	NCSR "Demokritos"
6)	Proukakis	Univ. Athens

I. Summary of Project Global Objectives and Achievements

The six participants to this Project represent five European countries that have not yet promulgated any recommendation, regulation or enforcement of an action level for indoor radon. Thus, no large statistical data base concerning the efficiency ("before/after" ratios) of various mitigation methods in actual houses are readily available from these countries.

On the other hand, a wealth of data have been gathered so far on the radioactivity of soils, soil emanation characteristics and high radon-prone areas during the Indoor Radon National Surveys of France, Italy, Portugal, Spain and Greece — some of these surveys still being under completion, however (e.g., France).

In many instances, it has been found that high radium concentrations in soils and unusual soil characteristics (e.g., high permeability) were not the exclusive explanations for abnormal indoor radon : building materials, indeed, may contribute substantially as well to the high levels of indoor radon disclosed in some dwelling houses.

Therefore, emphasis has been put upon the study of building materials and structural modules as a source of indoor radon (Spain and Greece). Typically, both the radium content and the exhalation rate of representative samples have been measured, yielding an evaluation of the emanation factor. The ability of various readily available covering materials to lower this emanation factor has also been thoroughly investigated (Portugal, Spain and Greece).

Other laboratory studies have turned on the measurement of the efficiency of potential radon barriers, that could be implemented in radon mitigation experiments. Both regimes of radon transport, namely diffusion and viscous flow, have been investigated, yielding experimental values of the diffusion and permeability coefficients (France).

At last, mitigation experiments at scale 1:1, either in test rooms (Portugal), or in actual houses (France, Italy and Portugal) are going on, that encompass most of the state-of-the-art radon mitigation strategies.

Head of project 1: Dr. J.C. Sabroux and Dr. M.C. Robé

II. Objectives for the reporting period

1) To study the permeability to radon of :

— concrete slabs of different make-ups and thicknesses ;
— concrete slabs with covering.

This study has taken advantage of the accurate and reproducible laboratory protocol established during the previous reporting period, and already used for testing the efficiency of various coatings, coverings, floorings and sealants as potential radon barriers.

2) To select, in a granitic region, a dwelling house with a high indoor radon concentration, and to identify radon sources and pathways in this dwelling house, by implementing the methodology of diagnosis that IPSN has developed.

3) On the basis of the comprehensive radon data recorded for the longest possible period of time and of the passive and active ventilation experiments, in this actual dwelling house, to determine the most appropriate radon mitigation techniques.

Objectives for the next reporting period.—

1) To implement the previously selected radon mitigation techniques in the dwelling house under study. The radon reduction strategy will incorporate some of the materials tested in the laboratory, and recognized for their high radon mitigation potential.

2) To resume, for an equivalent amount of time after implementing the radon reduction scheme, the comprehensive radon monitoring in the house already carried out before any mitigation attempt. The overall investigation will yield the yearly average efficiency of the mitigation approach selected.

III. Progress achieved including publications

Laboratory study.—

A laboratory apparatus was designed for studying, under controlled environmental conditions, the diffusion and permeation of radon through samples (20 cm diameter discs, 1 to 4 cm thick) of concrete. Samples of mortar for wall roughcasting were also studied.

The versatility of the apparatus (Figures 1a and 1b) allowed to measure the diffusion and permeability coefficients of a great deal of samples with different cement/water, cement/sand and cement/aggregates (if any) ratios. The effectiveness of several fillers and additives was also investigated. All samples were allowed to dry for 28 days.

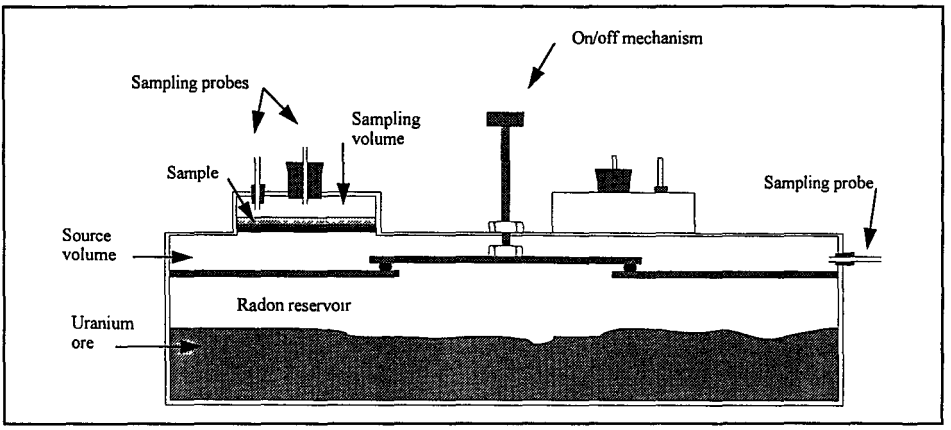


Figure 1a — Laboratory experimental setup for simultaneously measuring the diffusion coefficient of radon through three concrete samples.

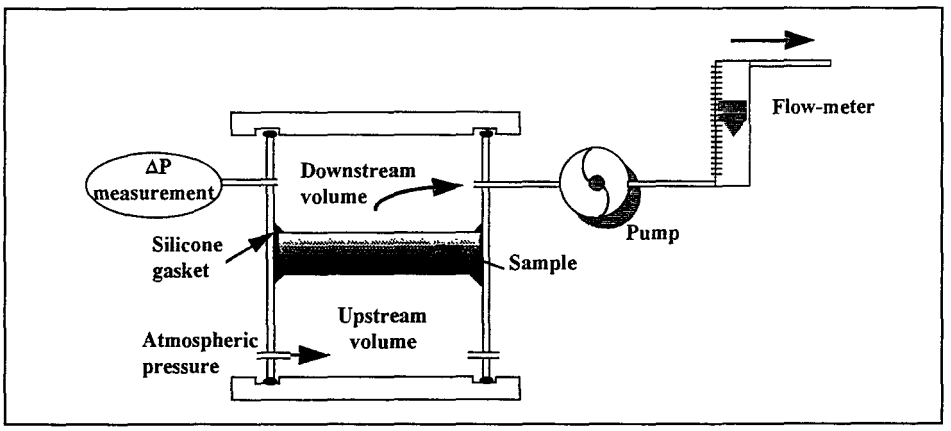


Figure 1b — Laboratory experimental setup for measuring the permeability coefficient of radon through one concrete sample at a time.

For selecting the most appropriate materials, the general guidelines were to raise the density and plasticity of the end product, and to lower its porosity and shrinkage upon drying.

By choosing appropriate ratios of constituents, and relevant fillers and additives, the diffusion coefficient can be kept below 10^{-5} $cm^2.s^{-1}$, both for concrete and mortar. On the other hand, the permeability turned out to be 3×10^{-18} m^2 and 4×10^{-15} m^2 for concrete and mortar, respectively.

The most effective composition of mortar and concrete, as selected from this laboratory study, will be implemented in the actual house under investigation, for a fully-fledged experiment of radon mitigation.

Field study.—

The building selected for the "before/after" study of radon mitigation is the city hall of a 3,000-inhabitant rural district of Britanny, a conspicuously granitic region of France. The advantage to choose an office and public-use building is that people spend less time in it, implying an alleviated nuisance from the radon monitoring system. Moreover, there is no real-estate transaction issues with such a building. At last, the human exposure in the premises can be readily compared with the radon standards set up for occupational exposure, e.g., in the uranium industry.

The building chosen is a granite freestone four-story house (including the attic and the half-buried basement). Part of the basement is taken up by a boiler room, with a hard-packed ground. The boiler, when in operation, induces a powerful periodical stack effect, that helped to identify unambiguously the soil gas as the main radon source. Radon is then drawn into the house along pathways that were traced using helium, during an experiment under various ventilation regimes.

Radon levels averaging 6,000 $Bq.m^{-3}$ and 400 $Bq.m^{-3}$ were measured in the boiler room and office rooms, respectively, with peak values higher than 20,000 $Bq.m^{-3}$ in the former and 1,000 $Bq.m^{-3}$ in the latter (Figure 2a and 2b).

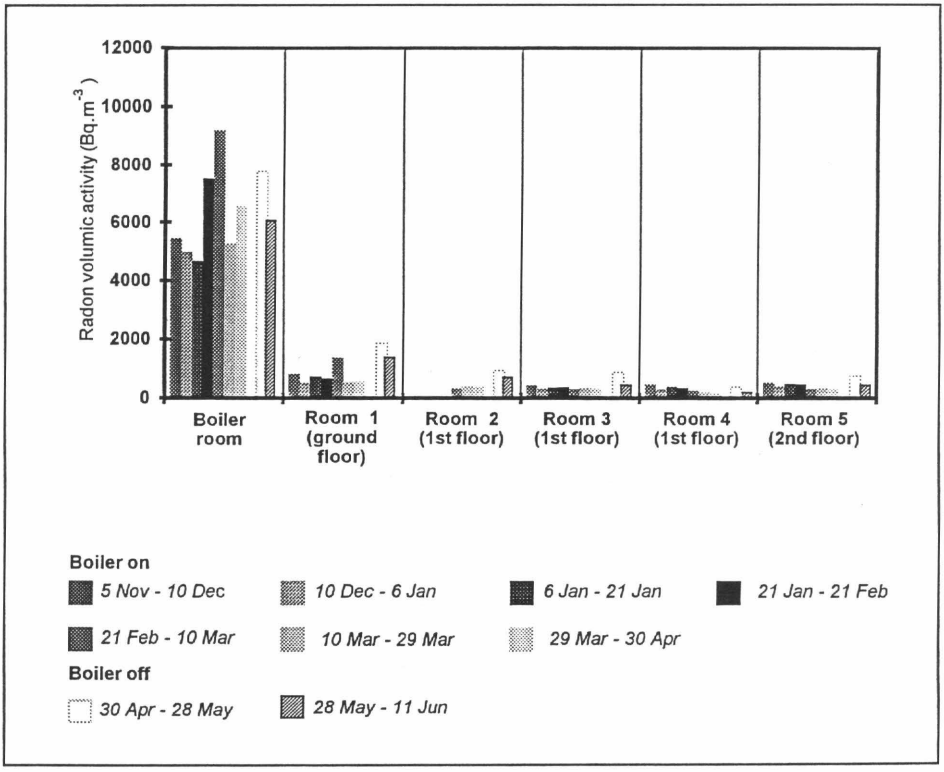


Figure 2a — Indoor radon measurements by means of track-etch detectors (KODALPHAs).

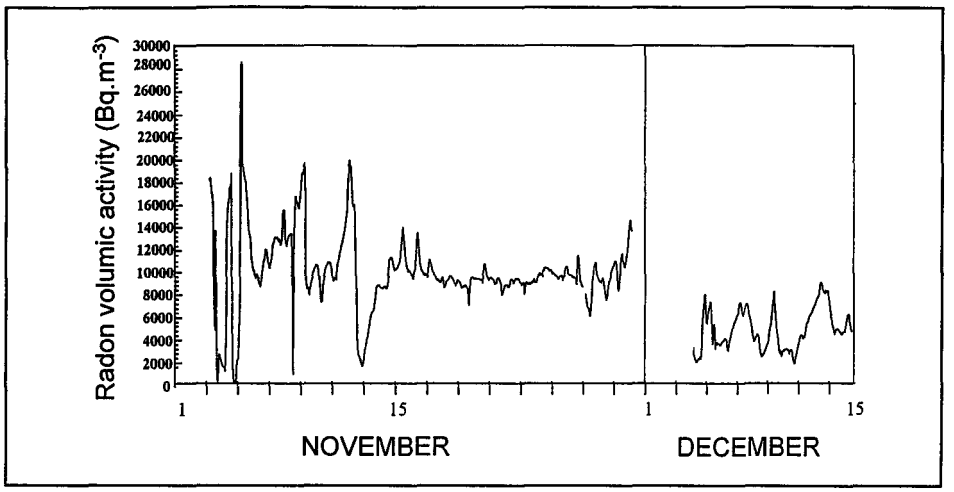


Figure 2b — Radon measurements in the boiler room by means of an ionization chamber.

Measurements currently carried out in the house include :

— radon indoors and outdoors, by means of ionization chambers, passive dosimeters (track-etch and electrets) and silicon detectors ;
— PAEC by means of site alpha dosimeters ;
— rate of emanation from the hard-packed surface of the boiler room (radon source) ;
— indoor and outdoor temperature and pressure.

We took advantage of the extension of the Project to carry on, for almost one year, with this set of measurements — before any mitigation measure. The seasonal effects on indoor radon concentration are thus satisfactorily delimited.

The mitigation strategy, to be implemented in October 1994, is centered on sub-slab ventilation. This stems from the comprehensive study of radon entry and distribution in the house, as described above, and from theoretical knowledge and assessment of the various techniques available so far for radon mitigation.

A concrete slab will be poured on a polymer membrane and gravel bed, to cover the packed-earth flooring the boiler room. Special care will be taken to avoid seams between the walls and the slab ; the drystone walls will be coated with mortar. Both pressurization and depressurization techniques will be tested.

Continuous and integrated measurements will resume after implementation of the mitigation techniques : they will proceed for six to eight months, in order to yield a thoroughly substantiated assessment of the radon mitigation efficiency of the countermeasures.

Head of project 2: Dr. G. Torri

II. Objectives for the reporting period

Two radon reduction systems have finally been selected for the application to the selected Italian typology of dwelling houses :

— sub-floor ventilation ;
— sub-soil depressurization.

In order to compare the efficiency of the radon reduction, an experimental system has been designed, and will be installed in the near future in at least three houses of various regions of Italy.

Further investigations have permitted to establish that the two houses in the Lazio region have been built on a big block of tuff, for which a relatively low permeability is presumed (10^{-15} to 10^{-16} m^2), while the soil beneath the third house (Friuli-Venezia region) is made up of pure soil mixed with gravel, which should be characterized by a comparatively higher permeability (10^{-10} to 10^{-11} m^2).

Objectives for the next reporting period.—

Implementation of the reduction system in, at least, three houses selected in different regions of Italy. Study *in situ* of the efficiency of the system .

III. Progress achieved including publications

The final version of the experimental apparatus has been designed.

The block diagram of the system to be installed in the three houses selected is shown in Figure 1.

A fan (250 W) is connected to the sub-floor space and to the sump through a system of pipes and manual valves. Two small tubes are inserted into the pipes venting the sump and the sub-floor space, and a third tube is directly connected to the house.

This system allows the measurement of the differential pressure between the house and the sump or the sub-floor space. It has been specially designed for offering the possibility to implement alternatively the sub-floor ventilation or the soil depressurization.

Moreover, it enables a comparative study of the efficiency of the two reduction techniques in the same house. Data will be also correlated with the permeability of the soil beneath each house.

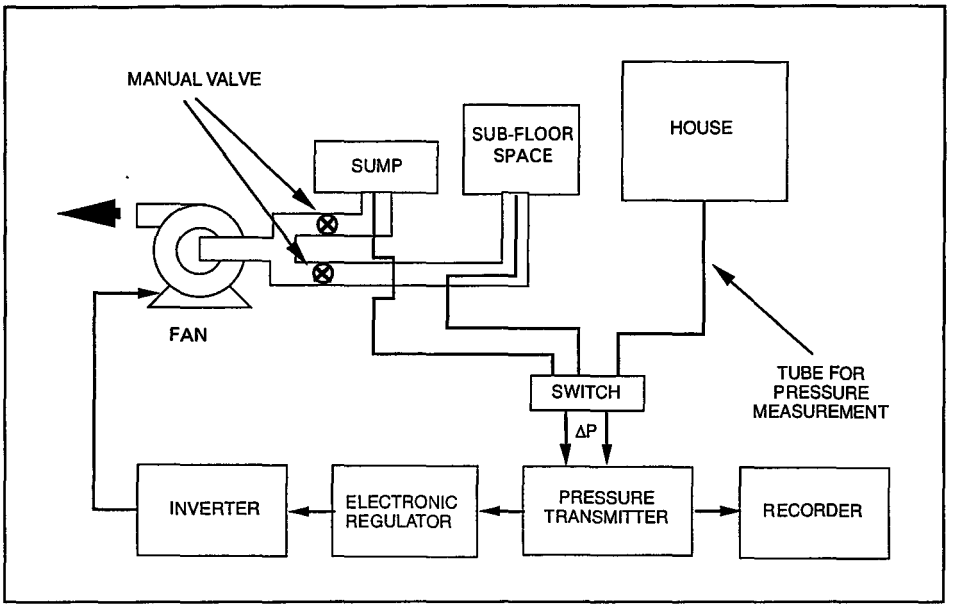


Figure 1 — The dual system for experimental radon mitigation in houses.

Furthermore, the system has been designed to study the efficiency of a given reduction technique with respect to the selected ΔP between the house and the sub-floor space or the sump. Indeed, the ΔP can be selected, and the power of the fan is automatically regulated in order to maintain the selected ΔP at its nominal value, by means of a differential pressure transmitter, an electronic regulator and an inverter.

Head of project 3: Dr. A. Ortins de Bettencourt

II. Objectives for the reporting period

1) To carry on with the measurements of indoor radon and radon daughters concentration under different kinds of natural ventilation.

2) To identify the main radon source term in some previously selected dwellings where high indoor radon concentrations were found.

3) To pursue the laboratory mitigation studies employing some usual building materials.

4) To verify the influence of the phosphogypsum used for coating the experimental laboratory walls. To cover the walls with different materials and to monitor their effectiveness.

Objectives for the next reporting period.—

1) To intensify the laboratory mitigation experiments, in order to study more building materials.

2) To perform an intercomparison of the experimental mitigation results with IPSN (France), on the same building materials.

3) To implement remedial actions in a real dwelling house, and to test their relative effectiveness. According to the experimental results, an overall assessment will be made of the cost-effectiveness of each remedial action.

III. Progress achieved including publications

Spot and integrated measurements of indoor radon and radon daughters have been carried on in the two previously selected buildings where high indoor radon concentrations were found. As one of the dwelling house is more readily available to apply future countermeasures, the measurements of indoor radon and radon daughters concentration have been intensified in this particular one.

The integrated indoor radon measurements in the latter ranged from a "low" 1,363 $Bq.m^{-3}$ up to 1,654 $Bq.m^{-3}$, with an average value equal to 1,540 $Bq.m^{-3}$.

Several of these measurements had been performed under different conditions of natural ventilation.

The results of the spot measurements of indoor radon and radon daughters concentration are summarized in Table I.

^{222}Rn (Bq.m^{-3})		PAEC (10^{-2} μJ.m^{-3})	
(1)	(2)	(1)	(2)
5,375	3,646	5.0	1.7
3,429	4,259	5.4	12.3
9,190	7,214	17.3	3.1
2,554	1,223	13.1	3.6
12,723	1,279	36.2	3.7
4,158	997	12.2	4.7
317	158	10.4	2.1
231	221	14.1	1.5

(1) measurements performed with the door and the window closed.
(2) measurements performed after having opened the door and the window for one to three hours.

Table I — Spot measurements of indoor radon and radon daughters.

From the results obtained so far, it can be observed that the indoor radon concentrations decreased down to a factor 10, with natural ventilation only.

Outdoor measurements had also been carried out around the house. The concentrations ranged from 16 Bq.m^{-3} up to 426 Bq.m^{-3}, the PAEC from 0.1×10^{-2} μJ.m^{-3} to 16.0×10^{-2} μJ.m^{-3}.

In order to identify the main indoor radon source term, several exhalation measurements from the walls and the wooden suspended floor had been performed. The results obtained are the following :

	^{222}Rn exhalation rate (mBq.m^{-2}.s^{-1})		
	minimum	average	maximum
Walls	0.2	0.5 ± 0.3	0.8
Floor	1.1	1.3 ± 0.2	1.5

Several soil samples collected in the neighborhood of the house were analyzed by gamma ray spectrometry : an average ^{226}Ra concentration of 723 ± 13 Bq.kg^{-1} was found. Exhalation rate measurements from the soil near the house were also performed, and the corresponding values range from 65 mBq.m^{-2}.s^{-1} to 129 mBq.m^{-2}.s^{-1}.

Concerning the laboratory mitigation tests, three materials have been studied until now : one concrete block and two vinyl materials used to cover the floor. With a 3 cm thick concrete block, a reduction factor of three was obtained. On the other hand, the vinyl materials tested show a significant mitigation potential, yielding a radon concentration reduction of two to three orders of magnitude.

The average indoor radon concentration measured in the experimental laboratory, before wall roughcasting with phosphogypsum, equals 50 $Bq.m^{-3}$.

After coating its walls with phosphogypsum (with a ^{226}Ra content of 370 ± 15 $Bq.kg^{-1}$), the new indoor radon concentration ranged from 250 $Bq.m^{-3}$ to 300 $Bq.m^{-3}$. Subsequently, the walls were painted with epoxy, and several integrated indoor radon measurements had been carried out. An average indoor radon of about 60 $Bq.m^{-3}$ was thus obtained, showing that the epoxy paint has a significant effectiveness.

Publications.—

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Faísca, M.C., Alves, J.G., Teixeira, M.M.R., Crispim, J.A. and Vaz Carreiro, J., 1994. Doses de radiação natural em grutas. Proceedings of the V° Congresso da Sociedade Espanhola de Protecção Radiológica. Santiago de Compostela, Spain, 26-29 Abril: 309-315.

Faísca, M.C. and Teixeira, M.M.R., 1994. Resistência através de barreiras ao transporte do radão. 9ª Conferência Nacional de Física, Covilhã, Portugal, Setembro.

Head of project 4: Dr. L.S. Quindós Poncela

II. Objectives for the reporting period

Within the framework of the Project, the global objectives to be developed by our Research Group are basically related to the contribution of building materials as a source of radon. The research rationale is to study, for these materials, effective remedial actions that will contribute to the decrease of radon in houses where the building materials have an important incidence. The specific objectives for the reporting period can be expressed as follows :

1) Development of protocols for the measurement, in porous materials, of physical parameters such as radium content, radon emanation factor, exhalation rate, diffusion coefficient, porosity and permeability. Intercomparison exercises for the measurement of these parameters — especially among the participants to the CEC Radiation Protection Research Action — are absolutely necessary for a good evaluation of the contribution, not only of building materials, but also of soil, as a source of indoor radon.

2) Study of the conditions under which permeability of concrete and granite may be responsible for the presence of high radon levels in houses.

3) Evaluation, in a test structure, of the relative contribution of granite — the main building material employed in an area of Spain identified as prone to high radon levels — to the radon concentration measured in houses.

Objectives for the next reporting period.—

1) Study in the laboratory of the efficiency of some caulking compounds, and of mitigation techniques for reducing radon levels, with and without the soil as a radon source.

2) Development of *in situ* measurements, in two selected houses, to evaluate the relevance and reliability of the above mentioned laboratory measurements.

3) Modelisation, by mathematical models, of the bearing of granite and concrete as a source of indoor radon, with and without appropriate remedial actions.

III. Progress achieved including publications

The presence of granite in houses affects not only the external gamma radiation, but also the indoor radon levels, especially when granite is used as a building material, or when the house is built over a granitic soil. At present, we have measured the radioactivity content in more than one hundred granite samples. Results are described in the paper entitled Natural radioactivity of cements and granites in Spain, where are also included the analyses of a total of 120 cement samples that cover about 95% of the Spanish brands of cement, and a small proportion of imported cements.

Throughout this reporting period, we have focussed our interest in developing a complete characterization of a selected set of granite and cement samples, all of them showing radium concentrations over 100 $Bq.kg^{-1}$. For that purpose, a total of 40 samples — 30 granites and 10 cements — have been studied. Radium content, radon emanation factor, exhalation rate as well as porosity and permeability are the analyzed parameters, in order to achieve this objective.

Concerning the radium content, a great deal of effort has been engaged in order to carry out an intercomparison exercise involving 13 different laboratories, most of them being involved in the CEC Radiation Protection Research Action. The edition of the final document reporting the results of the exercise is now ongoing. Two samples, phosphate and sand, were analyzed for their radium content by all the participants. Analysis of radium was achieved by using peaks of different energy that appear in the gamma spectrum. Table I displays, as an example, some of the results of the exercise. Moreover, a statistical analysis of the data has been carried out, in order to derive the best method for quantitative analysis of the radium content in mineral samples. This specific activity, not only in soils but also in building materials, is a key parameter for evaluating indoor radon in houses.

LABORATORY CODE	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12	E13
^{226}Ra phosphate (all lines)	*OUT*	*OUT*	*OUT*	—	*OUT*	*IN*	*OUT*	*IN*	*IN*	*IN*	*IN*	*IN*	*IN*
^{226}Ra sand (all lines)	*IN*	*IN*	*OUT*	—	*OUT*	*IN*	*OUT*	*IN*	*OUT*	*OUT*	*IN*	*IN*	*OUT*
^{226}Ra phosphate (609.32 keV line)	*IN*	*OUT*	*OUT*	—	*IN*	*IN*	*OUT*	*OUT*	*IN*	*IN*	*IN*	*IN*	*IN*
^{226}Ra sand (609.32 keV line)	*IN*	*IN*	*IN*	—	*IN*	*IN*	*OUT*	*IN*	*OUT*	*OUT*	*IN*	*OUT*	—
^{226}Ra phosphate (1,764.51 keV line)	*OUT*	*OUT*	*IN*	—	*OUT*	*IN*	*IN*	*OUT*	*IN*	*IN*	*IN*	—	*IN*
^{226}Ra sand (1,764.51 keV line)	*IN*	*IN*	*OUT*	—	*OUT*	*IN*	*OUT*	*IN*	*IN*	*OUT*	*IN*	—	—
^{228}Ac phosphate (all lines)	*IN*	*OUT*	*IN*	*IN*	*IN*	*OUT*	*OUT*	*IN*	—	—	*IN*	*OUT*	*IN*
^{228}Ac sand (all lines)	*IN*	*IN*	*IN*	*OUT*	*OUT*	*IN*	*OUT*	*IN*	*IN*	—	*OUT*	*OUT*	*OUT*
^{228}Th phosphate (all lines)	*OUT*	*IN*	*IN*	*IN*	*IN*	*OUT*	*OUT*	*IN*	*IN*	*OUT*	*IN*	*IN*	*OUT*
^{228}Th sand (all lines)	*OUT*	*IN*	*IN*	*IN*	*OUT*	*IN*	*OUT*	*IN*	*IN*	*IN*	*IN*	*IN*	*OUT*
^{40}K phosphate (1,460.75 keV line)	*IN*	*OUT*	*IN*	*OUT*	*OUT*	*IN*	*OUT*	*IN*	*OUT*	*IN*	*OUT*	*IN*	*IN*
^{40}K sand (1,460.75 keV line)	*IN*	*OUT*	*OUT*	*IN*	*IN*	*IN*	*OUT*	*IN*	*OUT*	*OUT*	*IN*	*OUT*	*IN*

TABLE I — Radioactivity measurements performed by 13 European laboratories the results of which are within the percentiles 25%-75% *(IN)* or outside this range *(OUT)*.

The technique for measuring the radon emanation factor in mineral samples can be readily implemented. Details of the procedure are described in the paper entitled A method for the measurement of the emanation factor of ^{222}Rn in small samples of porous materials. Basically, the rationale of the method we developed in our laboratory is equivalent to that of the commonly named "accumulation methods". The main innovation, however, is that the container used for enclosing the material sample to be tested is a hermetically sealed modified Lucas cell, which improves the sensibility of the method down to figures as low as 10^{-4} Bq.s^{-1} (0.36 Bq.h^{-1}). The cements analyzed, with radium concentrations higher than 100 Bq.kg^{-1}, yielded emanation factors within the range 0.6% to 5%. By using these cements and typical aggregates, we are now preparing concrete samples, in order to measure their emanation factor and compare it with the data for raw cements.

Specific intercomparison with two other laboratories has been carried out, showing the reliability of the technique

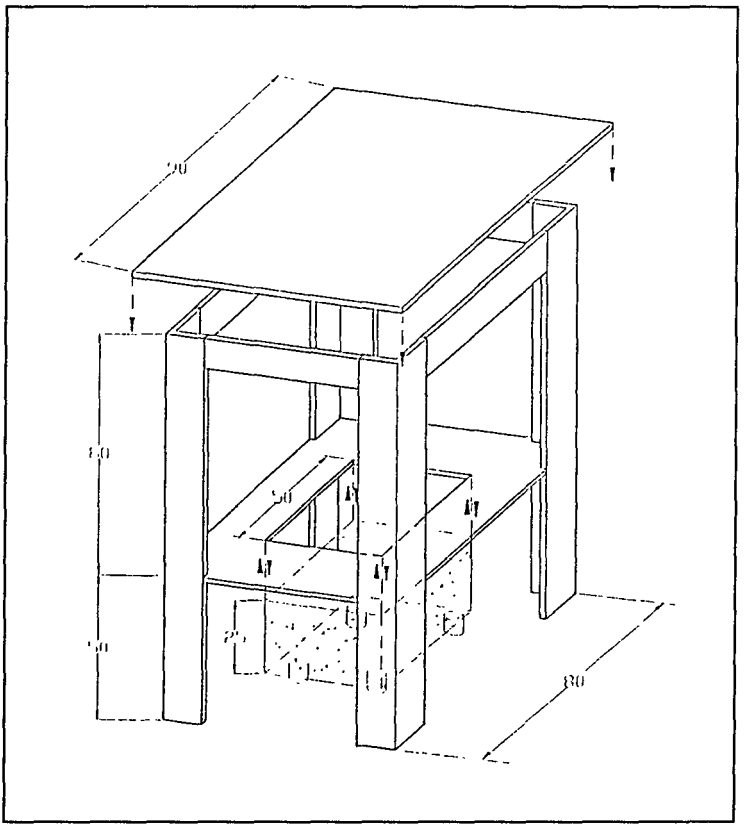

Figure 1 — The test "house". Effective volume : 1.875 m^3.
Volume of chamber with radon source : 0.040 m^3.
Effective exhalation area : 1.5 m^2.

The measurements of porosity and permeability of porous samples are also ready to be applied for any samples (e.g., cements or granites). For the cements, the porosity ranges from 40% to 60%, while for granites it is always below 2%. Regarding the permeability, typical values are 10^{-13} m^2 and 10^{-17} m^2 for cements and granites, respectively.

The low average value found for these last samples shows the weak incidence of the convective radon transport through this kind of material. In the test house built up in our laboratory, we are now studying the conditions under which the concrete samples referred to as above show a comparatively high transparency to radon.

Finally, we used the same technique for measuring the exhalation rate of 30 samples of granites : in this case, however, time was allowed for equilibrium to be reached in the cell. The results span from 0.01 $mBq.m^{-2}.s^{-1}$ up to 5.6 $mBq.m^{-2}.s^{-1}$; these data will be used for the completion of the next objectives. As a conclusion, we can consider that the objectives have been satisfactorily achieved, giving the possibility to propose standardized and reliable protocols for measuring the relevant parameters of radon escape and migration.

It is also possible to study, by simulation in a test house, the contribution of building materials to indoor radon by diffusion and convection mechanisms. Thus, we have built a granite "house" (Fig. 1) and studied the evolution of indoor radon, for different ventilation rates. In the experimental conditions adopted, the sole contribution of building materials is taken into account, and the results of measurements are being processed in our laboratory. Such a test house allows a radon source to be placed below ; it also makes it possible to generate and to measure pressure differences between the radon source and indoor air, for studying the convective processes for radon entry.

Publications.—

Quindós, L.S., Fernandez, P.L., Soto, J., Gomez, J. and Rodenas, C., 1994. Radioactivity in Spanish soils. *Health Phys.*, 66/2: 194-200.

Quindós, L.S., Fernandez, P.L., Soto, J., Rodenas, C., Gomez, J. and Arteche, J., 1994. Natural radioactivity of cements and granites in Spain. *Ann. Assoc. Belge Radioprotection.* 19/1: 289-298.

Quindós, L.S., Fernandez, P.L., Soto, J., *in press*. A method for the measurement of the emanation factor of ^{222}Rn in small samples of porous materials. *Radiat. Protec. Dosim.*

Head of project 5: Dr. P. Kritidis

II. Objectives for the reporting period

The principal objective was to continue the study of natural radioactivity and radon exhalation rate from the Greek building materials, including bricks and cements used in the Attica region. This was related to the refinement of the measuring procedures used, in order to improve their sensitivity, to reduce the contribution of the reverse entry effect, and to eliminate the counting of thoron.

A second objective was to establish a reliable system for the determination of the radon reduction properties of various covering materials (expressed in terms of diffusion coefficient, or of some attenuation factor).

Objectives for the next reporting period.—

— Completion of the measurements of natural radioactivity and radon exhalation rate from cement samples used in the Attica region.
— Measurement of the radon mitigation properties of various covering materials used in Greece, and evaluation of their effectiveness in terms of the reduction of average indoor radon concentrations in air, and of the related dose.

III. Progress achieved including publications

A modification of the laboratory method for the determination of radon exhalation rate has been developed and tested. The time schedules of the old and the new variant are as follows :

— old variant : radon collection (20-96 h), air circulation (5 min), equilibration (3 h) and measurement (1-3 h) ;
— new variant : simultaneous radon collection, air circulation and measurement (6-8 h).

The new variant allows to reduce up to tenfold the total measuring time, which makes possible the completion of the measurement within one working day (see Table I).

OLD VARIANT		NEW VARIANT	
sealing time	Rn exhalation rate	circulation time	Rn exhalation rate
20 h	2.0 $Bq.h^{-1}$	6 h	9.0 $Bq.h^{-1}$
70 h	0.7 $Bq.h^{-1}$	7 h	7.2 $Bq.h^{-1}$
96 h	0.6 $Bq.h^{-1}$	8 h	6.0 $Bq.h^{-1}$

TABLE I — Duration and detection limit of the radon exhalation rate measurements (old and new variant of the laboratory method).

The values shown above are determined on the basis of the typical background count rate of the total alpha-counting system, which is of the order of one c.p.m. The detection limit of the new variant is adequately low for most of the samples measured so far, but some of the samples with an extremely low emanation rate had still to be measured by using the old variant in its most sensitive mode.

The accumulation of thoron in the measuring system is eliminated by the use of a thin plastic cover, acting as a diffusion barrier. In order to test the efficiency of this barrier for thoron and its transparency to radon, a number of comparative measurements of sufficiently low-level samples have been done, where the "double counts" have been recorded separately. If the average counting rate is sufficiently low, e.g., below 0.5 c.p.m., the doublets of counts, time-spaced within $\Delta\tau < 0.3$ s, are practically all related to the alpha decays of ^{220}Rn and its daughter ^{216}Po (half-life : 0.15 s). The lack of double counts and the preservation of the radon-related count rate indicate an appropriate diffusion barrier.

		Specific activities Bq.kg^{-1}			Specific ^{222}Rn exhalation rate Bq.kg^{-1}.h^{-1}	Emanation coefficient %
		^{226}Ra	^{232}Th	^{40}K		
This study	range	25 - 83	35 - 65	540 - 1,060	0.0013 - 0.035	0.5 - 12.4
	average	35.6	51.1	732	0.008	2.8
	s.d.	12	8.9	158	0.009	3.2
Netherlands[1]	sample 1	39.1			0.0034	1.2
	sample 2	75.5				0.7
Belgium[2]	range				0.0008 - 0.0065	0.1 - 2.2
California[3]	average				0.0037	1
New Mexico[3]	average				0.013	6
Australia[4]	average	41	89	681		
Finland[5]**-1**	range	37 - 134	37 - 92	780 - 1,185		
	average	79.8	61.6	986		
Finland[5]**-2**	range	20 - 25	14 - 29	537 - 699		
	average	23	21	622		

[1] Dijk *et al.*, 1991 ; [2] Poffijn *et al.*, 1984 ; [3] Ingersoll *et al.*, 1983 ; [4] Beretka *et al.*, 1985 ; [5] Mustonen, 1984.

Table II — Measurements on bricks used in the Attica region, Greece, as compared with data on the same building material from other regions.

Publications.—

Savidou, A., Raptis, C. and Kritidis, P., *in press*. A study of natural radionuclides and radon emanation in bricks used in the Attica region, Greece. *Radiat. Protec. Dosim.*

Head of project 6: Prof. C. Proukakis

II. Objectives for the reporting period

The main objective of this project is the Investigation on the existence of a relation between ^{226}Ra content of building materials and radon exhalation rates. The principal steps to be investigated have been identified as follows :

— radon exhalation rate measurements, by α-spectroscopy, of prototype structural models (concrete slabs, brick and cement-brick walls) frequently used in Greek dwellings ;
— ^{226}Ra content measurements on specimens of the building materials to be used to construct the above mentioned modules, using γ-spectroscopy ;
— investigation on the existence of a correlation between the radioactivity of these modules, owing to their ^{226}Ra content, and the ^{222}Rn exhalation rate ;

Objectives for the next reporting period.—

1) Parametric study of the above correlations at various discrete steps, during the construction of the modules to be examined.

2) Study of the effects of various types of paints and ceramic wall tiles on the mitigation of the exhalation rate of structural modules.

3) Study of the effects of the ageing of the structural modules on the exhalation rate.

III. Progress achieved including publications

The project started on May 1993, and until now the following activities and results may be reported :

— sampling of building materials (cement, bricks, sand, gravel, marble powder, lime, gypsum, and pumice stones) produced by industries in various regions throughout the country ;
— pulverization of the sample below a 90 μm grain size, and preparation of specimens for γ-radioactivity measurements using high-resolution Ge detectors ;
— analysis of the samples (Table I) for natural radioactivity (^{226}Ra, ^{232}Th and ^{40}K) ;
— design and manufacture of a 1,200×1,200×700 mm (*ca.* 1 m^3) steel container to carry out radon exhalation measurements ; fitting of measuring and control instrumentation to this container ; software development for the monitoring and control of the container environmental parameters (temperature, pressure changes, humidity, aerosol concentration) ;

— α-spectrographic setup calibration for radon daughters analysis using a PIPS solid state detector and on-line software tools ; γ-spectrographic setup calibration for radon daughters analysis ;
— radon exhalation of raw materials (cements, bricks) and of structural modules (concrete slabs, brick walls).

Specific activity measurements.—

Specimens of cements originating from the three main Greek cement industries, of clay bricks fabricated by five big furnaces, that use raw materials mined at five different sites throughout the country, and also of other materials of — presumably — minor radiological importance, were collected for the purpose of this investigation. The specimens, if necessary, were dried at ambient temperature and pulverized to a grain size smaller than 90 μm. Finally, their water content was determined.

Each of the specimens was then used to fill two 0.280 liter plastic cylindrical boxes, about 72 mm in diameter and 70 mm high. The boxes were hermetically sealed, and covered with a film of epoxy resin to limit, as far as possible, any escape of radon. Thus, in each case, duplicate samples were subsequently analyzed.

The concentration of ^{226}Ra, ^{232}Th and ^{40}K in the samples were determined by high resolution γ-spectrometry. Detailed information concerning both the hardware and software configuration of the γ-spectrometer setups can be found in Simopoulos and Angelopoulos (1987). In order to allow for equilibrium of ^{226}Ra and ^{232}Th with their decay products, all specimens were analyzed at least three weeks after the boxes were sealed. The counting times were about 1.5×10^5 s (*ca.* 28 h).

The ^{226}Ra concentrations have been derived from the weighted average of the activities of two photopeaks of ^{214}Pb (295.2 keV and 352.0 keV) and of three photopeaks of ^{214}Bi (609.3 keV, 1,120.3 keV and 1,764.5 keV). In the case of ^{232}Th, two photopeaks of ^{228}Ac (338.4 keV and 911.1 keV) and the photopeaks of ^{212}Pb (238.6 keV) and ^{208}Tl (583.1 keV) were used in the same way. Finally, the concentration of ^{40}K was obtained from the single photopeak of this isotope (1,460.8 keV).

Table I summarizes the range (min - max) of the ^{226}Ra, ^{232}Th and ^{40}K specific activities in the building materials analyzed. The total uncertainty associated with these results, calculated from the systematic and random errors of measurement, ranges within 5% to 10% of the reported values.

For the sake of comparison, the same Table presents also relevant worldwide data compiled from Eichholz *et al.* (1980), Keller (1979), Porstendoerfer (1993), Sciocchetti *et al.* (1983), Stranden (1988) and UNSCEAR (1982). It is apparent from this Table that the natural radionuclides content of the various Greek building materials examined is comparatively very low. Only a few specimens of cement and clay bricks present a rather high ^{226}Ra specific activity, up to 147 Bq.kg^{-1} and 48 Bq.kg^{-1}, respectively.

MATERIAL	Sample size	Range (min - max) ($Bq.kg^{-1}$)			Worldwide range ($Bq.kg^{-1}$)
		^{226}Ra	^{232}Th	^{40}K	^{226}Ra
Cement	52	29 -147	13 - 30	172 - 331	6 - 196
White cement	10	14 - 26	7 - 13	5 - 67	
Clay bricks	5	28 - 48	37 - 56	727 - 895	4 - 200
Sea sand	6	7 - 13	8 - 16	145 - 302	
Sand	9	1 - 4	- 3	1 - 37	1 - 113
Marble powder	8			- 25	
Mosaic	7	1 - 3	1 - 3	- 23	15 - 55
Gypsum	6	6 - 17	- 10	5 - 40	4 - 330
Pumice stone	4	50 - 874	54 - 60	1,048 - 1,160	
Quicklime	2	9 - 32			8 - 20
Perlite	1	46	56	1,048	
Wall tiles	1	58	46	409	63 - 91

TABLE I. — Specific activity of natural radionuclides in Greek building materials.

Radon exhalation measurements.—

The radon exhalation rate of the building materials with a comparatively high radium content, as selected from Table I, was evaluated by using a specially designed 1 m^3 steel clad air-tight container, as introduced by Jonassen and McLaughlin (1980). The volume of the sample material — which could be as well a structural module such as a test wall or slab — should not exceed 10% of the total volume of the container, according to the suggestions by Poffijn *et al.* (1984) and Samuelsson (1987). The following important environmental parameters, which affect all exhalation experiments, are continuously monitored in the container by means of appropriate sensors and AD converters, interfaced with a PC : pressure changes (using a ± 50 mbar differential pressure transducer), temperature and relative humidity. An aerosol generator is also used to produce particles.

Three methods are employed for determining the radon concentration in the container environment. Two of these methods are based on grab sampling of a small portion (15-20%) of the container atmosphere through filters. The filters are subsequently analyzed, using :

— α-spectroscopic analysis of the radon daughters ^{218}Po and ^{214}Po, using a PIPS detector setup ;
— γ-spectroscopic analysis of radon daughters ^{214}Pb and ^{214}Bi using a Ge detector setup as introduced by Simopoulos and Angelopoulos (1990).

A third method, also introduced by Simopoulos and Angelopoulos (1990), was applied in order to conduct *in situ* radon progeny measurements inside the container, using a ^{241}Am doped 2"×2" NaI detector, which records the area encompassed by the 609 keV photopeak of ^{214}Bi.

For the calibration of the container, a Pylon 2000A (NIST cross-certified) source, with a nominal ^{226}Ra activity of 102.8 kBq was used. According to the systematic results already obtained in an aerosol-free environment, the efficiency of the facility, in terms of the fraction of radon progeny collected on the filter, was determined to be 0.1, with a r.m.s. error of about 15%. Experiments were conducted within a 0.1 - 30 Bq.m^{-3} range of radon concentration, and with a temperature and relative humidity in the 19 - 21°C and 30 - 40% range, respectively. Furthermore, preliminary results show that the efficiency is increased up to four times in the case of an aerosol-rich environment.

A least square fitting of the radon growth curve data from the examined materials was used to calculate their radon exhalation rate ; for this purpose, each of the specimens was enclosed in the container for a period of about 20 days. The estimated effective decay constants (Stranden, 1988) did not significantly differ from the decay constant of ^{222}Rn ; the total uncertainty associated with these calculations was about 25%. According to the results obtained, the cement specimens, with ^{226}Ra concentrations in the range 100 - 140 Bq.kg^{-1}, present exhalation rates between 15 μBq.kg^{-1}.s^{-1} and 20 μBq.kg^{-1}.s^{-1}. Pulverized to less than a 90 μm grain size, brick specimens, with ^{226}Ra concentrations in the range displayed in Table I, present exhalation rates between 3 μBq.kg^{-1}.s^{-1} and 10 μBq.kg^{-1}.s^{-1}.

Furthermore, two typical Greek structural modules, a clay brick wall and a concrete slab, with a 1 m^2 exhalation area, constructed from raw materials with the highest ^{226}Ra concentrations (Table I), were also tested. The exhalation rate of the wall, with calculated values of the ^{226}Ra content equal to 40 Bq.kg^{-1}, was 2 mBq.m^{-2}.s^{-1}, while that of the slab, with a ^{226}Ra specific activity equal to 26 Bq.kg^{-1}, was 3 mBq.m^{-2}.s^{-1}.

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Publications.—

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Louizi, A., Proukakis, C., Petropoulos, N.P., Anagnostakis, M.J., Simopoulos, S.E. and Angelopoulos, M.G., 1994. Measurements of natural radioactivity in Greek building materials. 1st Mediterranean Congress on Radiation Protection, Athens.

Louizi, A., Proukakis, C., Petropoulos, N.P. and Simopoulos, S.E., *in press*. Natural radioactivity content and radon exhalation rates of Greek building materials. Indoor Air, an Integrated Approach, International Workshop, Brisbane. *Proceedings*, Elsevier.

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Participation to intercomparison and intercalibration exercises.—

Intercomparison of gamma spectrometry measurements of natural radioactivity in soils, 1993-1994. CEC-RPP. L.S. Quindós Poncela, University of Cantabria, Spain.

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Progress Report

Contract: **F13P-CT920064d** **Sector: C12**

Title: Radon sources models and countermeasures.

1)	Miles	NRPB
2)	De Meijer	Univ. Groningen
3)	Majborn	Lab. Risø
4)	Wouters	CSTC/WTCB
5)	Ball	NERC
6)	Hubbard	SSI

I. Summary of Project Global Objectives and Achievements

The objective of the project is to enable individuals and governments to avoid excessive exposure to radon decay products by improving the understanding of the factors leading to high exposures. The probability of high radon levels occurring indoors is being mapped on a small and a large scale using information on geology and the results of measurements in the ground and in homes. Computer models of the movement of radon in the ground, building materials, sub-floor spaces and buildings are being developed and the results compared with the results from laboratory and field experiments. The effectiveness and reliability of various countermeasures are being assessed.

NRPB has studied the effectiveness of radon remedial measures in 345 dwellings and begun a long term study of durability. A large database of radon results in homes has been used to demonstrate a technique for mapping radon affected areas using only such results. KVI Groningen has completed the installation of its experimental set up of a large vessel filled with sand. Models to describe radon transport in the vessel were developed and comparison of the models and experimental data showed agreement within 15%. Risø has designed, constructed and validated a new field test structure for its studies of the transport of soil gas and radon. Forty-four soil probes have been installed and the effects of drawing air out of the soil have been examined. CSTC/WTCB has devoted this period to experimental in-situ studies in two dwellings. These have been aimed at improving the understanding of radon transport and determining which parameters need to be measured to decide on appropriate remedial measures. NERC has carried out measurements of soil-gas radon, Bi-214, Tl-208 and K over selected rock types in Somerset and Derbyshire. An initial assessment of the relationship between geological/geochemical parameters and house radon has been completed for Somerset. SSI has continued to monitor relevant parameters at its test house, adding wind speed, wind direction and barometric pressure to those measured. Three detectors of radon in soil gas have been installed at 1 m depth under the house and at either side.

During the study NRPB and NERC have collaborated on radon mapping by sharing data and calibrating equipment. A scientist from Risø spent 6 months working with the scientists at KVI on measurements and modelling of diffusive and advective radon transport in soil. CSTC/WTCB has carried out modelling calculations on the experimental set-ups at SSI and other laboratories. The group met during the Rimini radon workshop to discuss collaborative work, and intends to meet again at Risø in the next reporting period.

Head of project 1: J C H Miles

II. Objectives for the reporting period:

The objectives for the reporting period were: (1) to continue to build up a database on the effectiveness of different types of radon remedial measures installed in homes and to analyse the data to determine the most appropriate measures for different types of construction and different radon levels, (2) to start a study of the durability of remedial measures in homes by making annual measurements of radon levels in a sample of homes, (3) to use a large database of radon measurements in homes to develop and test techniques for mapping radon affected areas accurately and economically.

The objectives for the next reporting period are to continue to collect and analyse data on the effectiveness and durability of radon remedial measures, to identify house types for which existing remedial measures are insufficiently effective or reliable, and to improve techniques for reducing radon levels for these houses. The effects of smoothing radon potential maps will be examined, and population density maps will be used with radon potential maps to identify areas where large numbers of homes need to be remedied.

III. Progress achieved including publications:

In the UK, over 16,000 homes with annual average radon concentrations exceeding the UK Action Level of 200 Bq m^{-3} have been discovered. Some 600 householders who have taken action have sought confirmatory measurements from NRPB. Results for 345 such homes are available and are shown in Table 1. The ratio of the seasonally-adjusted radon measurements before and after remedial action are given. The arithmetic mean (AM) of the reduction factors can be misleading, since it is much affected by the occasional very high value. The geometric mean (GM) is not so distorted, and is often a better indication of the typical reduction to be expected.

A range of techniques is effective in reducing radon levels in homes. Although disappointing results can occur with all methods, such occurrences are rarer for sump systems than other techniques. Only sump systems have produced reductions in radon level by two orders of magnitude. The least disruptive remedial actions are additional underfloor natural ventilation for suspended floors and positive ventilation for homes with slab on grade floors. Although appreciable reduction factors can sometimes be achieved with these methods, reductions are more likely to be low. Likewise, floor sealing alone can sometimes be fairly effective, but is time-consuming and labour-intensive, particularly so where large reductions are required and very expensive if carried out by a contractor.

It is important that the effectiveness of remedial action be maintained well into the future. A number of remedied homes are being remeasured annually to determine the durability of the remedies: results for the first year follow-up measurements are given in Table 2. The ratio of the radon level measured in 1993 to that immediately after remedial action is used as an indication of durability: values of 1.0 or less indicate durability whereas values above 1.00 indicate a deterioration.

The indication from Table 2 is that effectiveness can be maintained, although the duration

of the study so far is quite short. With sump systems, eleven cases for which results are available showed no change or an improvement. Three sump installations had an apparent decrease in effectiveness, but these had very low radon levels around 40 Bq m^{-3} after the systems were installed, whereas the pre-remedial radon levels were 1600 Bq m^{-3}, 1100 Bq m^{-3} and 780 Bq m^{-3}.

Table 1. Effectiveness of radon remedies

METHOD	N	REDUCTION FACTOR			
		AM	GM	Min	Max
Positive ventilation	70	3.2	2.2	1	24
Additional natural ventilation	25	2.3	2.0	1	6.0
Additional natural ventilation of underfloor void	46	3.1	2.1	1	17
Mechanical ventilation of underfloor void	14	4.0	2.2	1	17
Sealing only	40	2.3	1.4	1	32
Membrane covering floor	21	2.2	1.9	1	6.5
Sump	159	16	8.3	1	110
Sump with other method(s)	33	11	5.0	1	64
Combination of methods, no sump	36	3.6	2.6	1	14

Table 2 Durability of radon remedies

METHOD	N	DURABILITY	
		AVERAGE	RANGE
Positive ventilation	6	0.97	1.37-0.37
Additional natural ventilation of underfloor void	5	1.41	2.79-0.45
Mechanical ventilation of underfloor void	4	1.02	0.64-1.33
Sealing only	3	1.24	1.61-0.64
Membrane covering floor	1	1.13	-
Sump	15	1.01	2.1-0.72

A database of 62,555 radon results in southwest England was used to determine whether the data within 5 km grid squares was consistent with lognormal distributions. Standard statistical tests gave conflicting results, so an alternative and more appropriate test was

carried out. The geometric mean (GM) and geometric standard deviation (GSD) were calculated for each distribution and used to calculate the expected fraction of homes above the Action Level. This was compared directly with the measured fraction above the level. The obvious way to calculate the GMs and GSDs is to take logarithms of all results for a grid square and use standard formulae to calculate mean and standard deviation. However, even if the underlying distribution of radon levels in homes is lognormal, there are two factors which distort the observed distribution and lead to erroneous estimates of GM and GSD. These are the contribution of outdoor radon to indoor levels and random uncertainties in radon measurements.

The distortion of the distribution caused by outdoor radon was removed by subtracting the outdoor radon concentration (4 Bq m^{-3}) from all results. If the GM and GSD of the resulting distribution are calculated using the standard formulae, random normally distributed errors in radon measurements will affect the values found. An alternative more robust method for estimating GM is to sort all of the results by size and take the median, the value half way down the list. Similarly to estimate GSD the radon result 84.15% down the list may be determined (corresponding to 1 GSD above the mean) and divided by the median. The values of GM and GSD determined using this technique should be less affected by the distortion caused by random errors. Determination of the GM and GSD by this method is referred to as the sort technique below. In order to compare the accuracy of the sort technique with the use of the standard formulae, a lognormal distribution was generated and the two techniques applied. It was shown that the standard formulae underestimated the GM and overestimated the GSD. In order to avoid inaccuracies introduced by random errors in measurement, the sort technique is used below to estimate GM and GSD.

The 62,555 radon results for the two counties had outdoor radon subtracted, were corrected for seasonal variation and selection bias, and were grouped by 5 km grid square of measurement. The fraction of the results in each grid square exceeding 200 Bq m^{-3} was determined directly from the data and compared with the fraction estimated using the techniques described above. The results showed that the lognormal model, used in this way, is a very good estimator of the fraction of the housing stock above a threshold over a wide range of values.

If the lognormal model is to be used in practice for radon mapping, it is necessary to estimate the GM and GSD for grid squares in which few data are available. It was shown that the variation of GSD with GM was small, implying that the same GSD may be used for all squares. Tests confirmed that using average GSD did not introduce any bias into the estimates of the fraction of the housing stock above 200 Bq m^{-3}.

In order to estimate the GM where few data are available GMs were smoothed between squares. To derive the smoothed estimate for a grid square a weighted mean was taken of the values for the square and the eight surrounding squares. To discover whether this procedure improved the estimate of the GM in cases where the data were sparse, a subset of the data was used. For each grid square 5 of the available results were selected at random, and the GMs estimated. The correlation coefficient between these GMs and the GMs of all data for the corresponding grid squares was calculated. This procedure showed that smoothing GMs where results are sparse did indeed improve the correlation with the GMs from the full data set.

The following conclusions were drawn from the results of the study so far:

1. The distributions of radon levels in 5 km grid squares are consistent with the lognormal distribution.

2. It is essential that the measurements be carried out using an appropriate selection of homes, location of detectors, duration of measurements, and accuracy of results over a wide range of concentrations.

3. Results need to be corrected to take account of outdoor radon levels, bias in selection of homes, and season of measurement.

4. The sort technique is more accurate in estimating the parameters of the distributions than the standard formulae where sufficient data are available.

5. In areas where there are too few results to allow GSD to be estimated directly, the mean GSD from squares with enough results may be used.

6. Smoothing GM values with those in adjacent squares improves the accuracy of the estimated GM in squares with few results.

Publications:

Cliff, K. D., Green, B. M. R. and Lomas, P. R. Domestic radon remedies. Radiation Protection Dosimetry, 45, 599-601 (1992)

Cliff, K. D., Naismith, SP, Scivyer, C, and Stephen, R. The efficacy and durability of radon remedial measures. Presented at the First International Workshop on Indoor Radon Remedial Action, Rimini, Italy, June 1993.

Miles, JCH. Mapping the proportion of the housing stock exceeding a radon reference level. Presented at the First International Workshop on Indoor Radon Remedial Action, Rimini, Italy, June 1993.

Cliff, K. D., Miles, JCH and Naismith, SP. False positive and false negative results due to uncertainties in seasonal correction factors. Presented at the First International Workshop on Indoor Radon Remedial Action, Rimini, Italy, June 1993.

Head of project 2: R J de Meijer

II. Objectives for the reporting period:

The objective of this project is to obtain a better understanding of radon in soil and in particular transport from soil to crawl space. More specifically, the objectives for this reporting period are to measure and model diffusive and advective radon transport for a cylindrical column of soil (dry homogeneous sand) and without a crawl space added to the experimental situation.

The objective for the next reporting period is to gradually increase the complexity of the situation. The first step will be the addition of a crawl space to the experimental set-up. Thereafter the effect of time-dependent pressure variations over the sand-column on the radon entry in the crawl-space will be assessed. Also the influence on radon entry of, height and ventilation of the crawl space, a water level in the sand and covering the surface of the sand will be studied. After each increase in the complexity of the situation models will be developed or adjusted and in case model calculations agree with measurements the complexity is increased further.

II Progress achieved including publications

Introduction

In the initial stage of this project a large vessel (diameter and height 2 m) was built and installed. The vessel is filled with sand and nine multi-functional probes allow measurement of pore water content, air permeability of the sand and radon concentration in the soil gas at different depths under the sand surface. A crawl space with underlying soil can be simulated by closing the vessel with a lid that can be adjusted in height. For the study of advective radon transport a perforated circular box is placed in the sand close to the bottom of the vessel.

Assessment of parameters and measuring procedures

To assess the homogeneity of the sand column in the vessel measurements of pressure fields inside this column were made. It was found that deviations from ideal homogeneity were less than approximately 15%.

To measure permeability of the sand with the probes, the shape factors of these probes have to be known. We determined these shape factors experimentally and theoretically and found good agreement. From theoretical considerations we also concluded that the sand sampled in a permeability measurement is located in a sphere with radius of approximately 20 cm, as far as we can judge at the moment this resolution is sufficient.

To measure the radon emanation factor of the sand a method was developed to deduce this factor from an experiment in which radon and thoron exhalation of a sample is measured simultaneously. This method was applied to determine the radon emanation factor of the sand (in dry condition) used in the vessel. The first tests to extend this method to measure

the radon emanation factor as a function of moisture content of the sand are now underway.

Procedures to sample soil gas through the probes and to measure radon concentration in the samples were developed. It is estimated that radon concentrations in these samples can be measured within an uncertainty of less than 6-7%.

Measurements of diffusive and advective radon transport

The sand column was flushed by inducing a large flow of radon-free air through the perforated box. Radon concentrations at all nine probes were measured after 7, 31, 55, 79, 103, 127, 155 and 500 hours after stopping the flow. The results of these measurements (Figure 1) show that equilibrium is reached about 150-200 hours after flushing the column.

Steady-state radon concentration profiles were also measured with air flows of 0.1, 0.2, 0.4, 0.8, 2.2, 6.5, and 10 L min^{-1} (Figure 2). In comparing these profiles with the steady-state profile without air flow it is clear that even a very small air flow drastically alters the radon profile in the sand. Instead of a gradual decrease in radon concentration from bottom to surface of the column, a maximum in the concentration is found of which the position shifts to the surface of the vessel for higher flow rates.

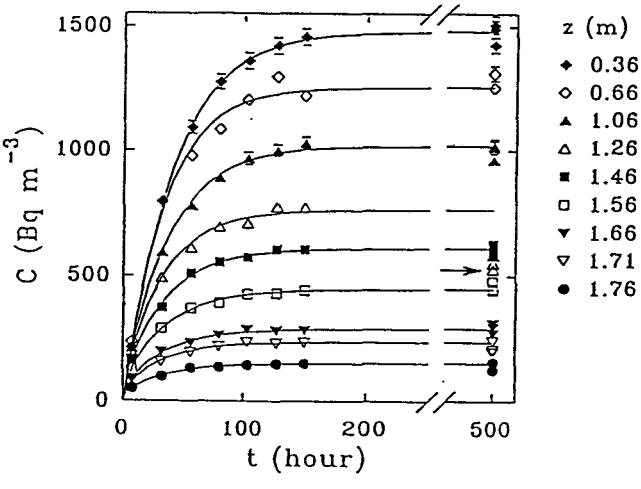


Fig. 1: Radon concentration C(Bq m^{-3}) as a function of time t (hour) after flushing for nine different probes. z-coordinate gives position of probe above the bottom of the vessel. Markers indicate experimental data. Error bars denote statistical errors. Solid lines result from least-squares fits (see text).

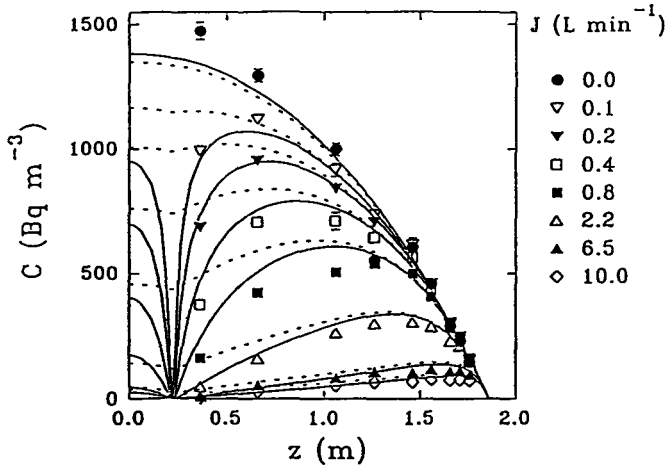


Fig. 2: Radon concentration C(Bq m^{-3}) as a function of z-coordinate for different values of the air flow J. Data points (indicated by markers) and error bars are mean values and standard deviations of three measurements. Dotted and solid lines represent curves as calculated from the 1D- and 2D numerical model, respectively.

Models for radon transport

Various models of increasing complexity were developed to describe radon transport in the vessel. In all models it is assumed that both the adsorption of radon to the sand grains and mechanical dispersion is negligible and that the sand may be considered as an isotropic dry porous medium.

With these assumptions the time-dependent radon transport equation has been solved analytically in one dimension in case no advection (no air flow) is present and with boundary conditions appropriate for the vessel geometry (1D analytical model).

For the case that advection is present the time-dependent radon transport equation has been solved with numerical procedures for both one (axial) and two (axial and radial) dimensions (1D and 2D numerical model).

Comparison of models and measurements

To assess if the experimental data in Figure 1 is described by the 1D analytical model this model was fitted with a least-squares procedure to the data points of each individual probe, using the tortuosity and emanation factor as free parameters. Figure 1 shows that the model describes the data quite good.

The steady-state profiles corresponding to the different air flows were also calculated with the 1D and 2D numerical model using the fitted values for the tortuosity and emanation factor. Figure 2 clearly shows that the 1D numerical model overestimates the data points, the 2D-model however reproduces the data within 15%.

Conclusions and outlook

From the agreement between model calculations and experimental data we conclude that for this simple geometry and soil type the 1D analytical model that describes diffusive radon transport is consistent with measurements. For combined diffusive and advective transport radon concentrations can be calculated with the 2D numerical model within an uncertainty of 15%. To further validate and develop our models it is planned to perform measurements in more complex situations.

Publications

1 van der Graaf, E.R., Heijs, S., de Meijer, R.J., Put, L.W. and Mulder, H.F.H.M. *A facility to study transport of radon in soil under controlled conditions*, Radiation Protection Dosimetry, 223-226, 1992.

2 van der Graaf, E.R. and de Meijer, R.J. *Progress report Radon Transport in Soil, phase 2, July 1, 1992 - March 31, 1993*, KVI internal report R45, April 3 1993.

3 van der Spoel, W.H. *Modelling of radon transport in the KVI radon vessel*, KVI internal report R57, July 27 1993.

4 van der Graaf, E.R., Witteman, G.A.A., van der Spoel, W.H., Andersen, C.E. and de Meijer, R.J. *Measurements on, and modelling of diffusive and advective radon transport in soil*, presented at the First International Workshop on Indoor Radon Remedial Action, 27 June - 2 July, 1993, Rimini, Italy, KVI internal report R50, May 1993.

Head of project 3: Dr. B. Majborn

II. Objectives for the reporting period

- To establish a new test structure for studies of soil-gas and radon transport.

- To carry out steady-state experiments at the test structure.

Objectives for the next reporting period

- To continue the experimental investigations, the site-characterization and the modelling of the test structure.

- To compare the modelling results with data from real houses. This work will be carried out in close collaboration with SSI (Sweden) and KVI (the Netherlands).

- To apply the numerical model to investigate the influence of building and soil related factors on radon entry into real houses with an emphasis on slab-on-grade houses.

III. Progress achieved including publications

This reporting period has mainly been devoted to designing, constructing, and validating the new test structure and to start the measurement programme. In addition, the collaboration with KVI (the Netherlands) has been intensified through a 6 months postdoctoral stay at KVI by one of the investigators from Risø.

Test structure design: The new test structure has been established at a field site at Risø National Laboratory at a location near to the structure used in the previous CEC funding period. The main difference between the new and the old structure is that the new structure is equipped with concrete footing and a concrete slab, thereby simulating a slab-on-grade foundation more closely. In addition, a larger number of probes have been installed in the soil below and around the new structure for mapping of pressure couplings and radon concentrations.

Figure 1 shows a cross-sectional view of the new quadratic test structure. The side length is 2.6 m and the footing extends approximately 0.75 m below the surface of the soil. A 40 litre cylinder (measuring chamber) is bolted to a steel plate that has been poured into the center of the slab. Soil gas and radon enter the cylinder through a 9.5 cm hole in the bottom. A capillary breaking layer of highly permeable gravel is located below the slab. A shelter for

Publications:

Andersen, C.E., Søgaard-Hansen, J. and Majborn, B. *Radon entry into a simple test structure.* Radiat. Prot. Dosim. 45(1-4) 407-410 (1992).

Andersen, C.E., Søgaard-Hansen, J. and Majborn, B. *Soil-gas and radon entry into a simple test structure: Comparison of experimental and modelling results. Workshop contribution and additional figures.* Risø-I-716(EN). Internal report, Risø National Laboratory, Roskilde, Denmark. Presented at the First International Workshop on Indoor Radon Remedial Action, Rimini, June 27 - July 2, 1993.

van der Graff, E.R., Witteman, G.A.A., van der Spoel, W.H., Andersen, C.E., de Meijer, R.J. *Measurements on, and modelling of diffusive and advective radon transport in soil.* R50. Internal report, Kernfysisch Versneller Instituut, Groningen, The Netherlands. Presented at the First International Workshop on Indoor Radon Remedial action, Rimini, June 27 - July 2, 1993.

Figure 1. Cross-sectional view of the new test structure. The probes located below the slab are named M20 to M31.

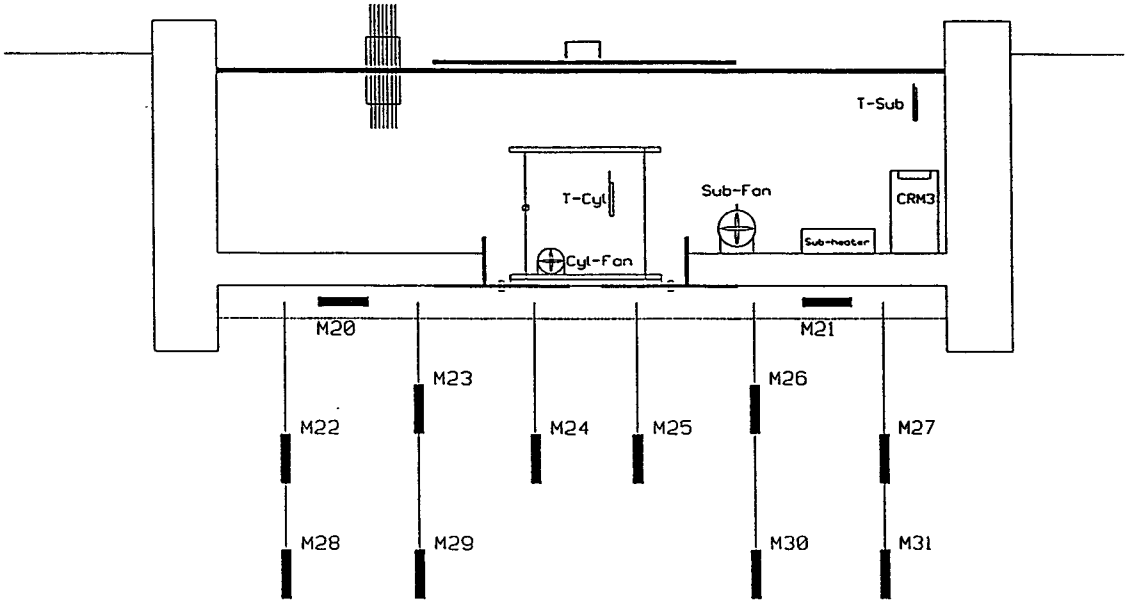


Table 1. Results of the measurements of the soil-gas entry rate into the test structure per Pascal depressurization (i.e. the inverse flow resistance). The results have not been adjusted for differences in temperature and atmospheric pressure.

Date	Inverse flow resistance L min^{-1} Pa^{-1}
October 5, 1992	0.25
October 8, 1992	0.27
December 29, 1992	0.11
January 15, 1993	~0
April 7, 1993	0.26
May 14, 1993	0.33
June 14, 1993	0.33
July 5, 1993	0.34
August 11, 1993	0.11

Table 2. Results of the scintillation cell measurements of radon concentrations in the substructure, the cylinder, and the probes named M20 and M21 (located in the highly permeable gravel below the slab as shown in Figure 1). The indicated uncertainties are observed standard errors, and N is the number of measurements. Column 2 gives the flow of soil gas that is drawn into the cylinder by the mass-flow controlled pump.

Period	Flow L min^{-1}	Substructure kBqm^{-3}	Cylinder kBqm^{-3}	M20 kBqm^{-3}	M21 kBqm^{-3}
Nov. 1992- March 1993	0	5.5±0.4 (N=10)	40±3 (N=11)	48±4 (N=12)	47±3 (N=14)
April - May, 1993	20	5.1±0.1 (N=3)	4.3±0.2 (N=4)	3.8±0.2 (N=6)	4.1±0.2 (N=6)
May - June, 1993	0	5.1±1.2 (N=3)	40±4 (N=3)	47±2 (N=6)	42±3 (N=5)
July - August, 1993	5.5	6.7±0.3 (N=4)	13±1 (N=4)	22±1 (N=8)	15±1 (N=8)

Head of project 4: P. Wouters

II. Objectives for the reporting period:

The CSTC-WTCB contribution to the project is to study, from theoretical and experimental point of view, the role of ventilation and air flow patterns in the framework of radon, with a particular attention to radon reduction techniques related to these parameters. This includes model calculations and in-situ experiments, some of them in collaboration with other participants to the project. The objective for the reporting period was to deal mainly with the in-situ experiments. In particular, the intention was to consider, in the framework of case studies, the question of the definition and evaluation of radon remedial actions and the problem of the choice of the parameters to be measured in order to define appropriate remedial actions.

During the next period, data analysis and model calculations will be carried out. The set of data concerning climatic, radon and tracer gas measurements performed as a case study in a dwelling of Visé (see a. hereunder) will be analyzed and discussed. On the other hand, the model calculations announced in the work programme of the present contract will be performed. The TRISCO model should be used for the prediction of pressure fields and air flows from the ground into a building, in collaboration with SSI. The VENCON model will be used to study the influence of climatic parameters on the air flows and pollutant transport in buildings, and on the performances of radon reduction techniques based on ventilation.

III. Progress achieved including publications:

The last months were exclusively devoted to experimental in-situ studies. Various measurements were performed in two dwellings located one in Court-St.-Etienne and the other one in Visé. In addition of the achievement of a better understanding of the radon transport, these two studies deal, among others, with the questions of the choice of the parameters to be measured in order to define appropriate remedial actions, and of the applicability of the soil depressurization technique in "difficult" situations.

a) A case study in Visé

The "Visé dwelling" is a building in which concentrations of several thousand Bq/m^3 were measured in occupied rooms. It has two well separated cellars covering 30% of the total ground floor area. The cellars probably play an important role: there exists no ground covering ("cellars on dirt") and there are many entry routes connecting these cellars to the rest of the building.

The decision of evaluating first the SSD technique in this dwelling was taken after a series of airtightness evaluations. The soil depressurization system was connected to the soil of one of the basements. The pressure differences between the soil and the dwelling indoor were measured in the second cellar. The use of this technique was a success in spite of a weak pressure field extension and in spite of the absence of ground covering at the place where the system was installed. More details concerning this study may be found in

references 3. and 4.

The two basements play an important role in this building. However the relative importance of each basement as a source-room for the rest of the dwelling is probably different for each one and will probably depend on the climatic conditions. Moreover, the conclusion concerning the effectiveness of the SSD technique even when very low soil/indoor pressure differences are measured in the second basement may not be valid if this basement is not a source room for the building. Therefore, it was decided to perform tracer gas measurements in order to confirm the conclusion concerning the effectiveness of the soil depressurization method in "difficult" situations and to better understand the radon transport in the dwelling. Two tracer gases were used (N_2O and SF_6), one in one of the basements, the other one in the second basement. The concentrations of each one of the two gases were measured in the basements and in each room, in function of time, together with climatic parameters (wind velocity, wind direction and temperature). In parallel, radon concentrations were measured in the rooms. The measurements covered a period of one month and are now terminated. The data analysis will start in the near future.

b) A case study in Court-St.-Etienne

The "Court-St.-Etienne dwelling" is another building with high radon concentrations (several thousand Bq/m^3 in occupied rooms).
Before contacting us, the inhabitants installed a "subslab natural ventilation" system, without success. We used the pipes installed by the occupants to install and evaluate the active SSD method, in spite of the existing difficult conditions: very bad airtightness of the slab, clayey soil, installed suction pipes occupying an unfavourable position. The soil/house pressure differences measured far from the suction pipes indicated that the pressure field extension was weak. In spite of that the use of the SSD technique was a success. The measurements are terminated and the data analysis is completed. A CSTC-WTCB internal report concerning this study is being prepared.

Publications:

1. P.Cohilis, P.Wouters and P.Voordecker, "Radon reduction in buildings: the case of two Belgian schools", to be published by ASHRAE as part of the International Conference on Building Design, Technology & Occupant Well-Being in Temperate Climates, Brussels, Belgium, 1993 (was written under the present contract but concerns a study finished at the very end of the 1990-1992 contract)

2. A.Poffijn, G.Eggermont, S.Hallez, P.Cohilis, "Radon in Belgium: Mapping and mitigation in the affected area of Visé", presented at the First International Workshop on Indoor Radon Remedial Action, Rimini, Italy, June 1993

3. P.Cohilis, A.Poffijn, P.Voordecker, G.Meesen, P.Wouters, "Radon reduction in buildings: selection and evaluation of a mitigation technique. A case study in Visé (Belgium)" , CSTC-WTCB, internal report, 1993 (distributed at the CEC Contractor's Meeting in Rimini, 30 June 1993)

4. P.Cohilis, A.Poffijn, P.Voordecker, G.Meesen, P.Wouters, "Selection and evaluation of a radon mitigation technique: a case study in Visé (Belgium)", selected for presentation at the Euregional Symposium on Radon, Liège, Belgium, 4-6 November 1993

Head of project 5: Dr. T. K. Ball

II. Objectives for the reporting period

The aims and objectives of the project were to develop radon potential mapping techniques. To investigate the roles that existing geological and geochemical data sets; new measurements of soil and bed-rock radioelement concentrations; and in particular the determination of soil gas radon levels, might have to the efficient recognition of zones of high radon values in houses. Previously field work has been undertaken on Geological Survey sheets. In this leg of the project, data was to be collected on county-wide basis. Field work was to undertaken in Somerset in 1992, and in Derbyshire during the summer of 1993. Because of the large housing data-set for these two counties, the possibility of determining a more precise relationship between geology and house measurements was to be investigated.

For the next year, the project is directed towards understanding the geological controls on radon potential. The housing stock for the whole of the county of Derbyshire is being investigated by the NRPB. The logistics of extending out from the well surveyed Chapel en le Frith area, mapped in the first round, to include similar lithologies in neighbouring areas and to develop procedures for covering larger areas more efficiently, are being studied. Hitherto field areas have been chosen to combine a high density of house measurements with geological features typical of an European context. The glaciated areas are characterised by being mainly glacially eroded, with relatively minor areas of glacial deposition. We propose to extend our studies to areas with more glacial deposits, typical of those areas in northern Europe which were also near the limit of glaciation.

III. Progress achieved including publications.

The main objective over the reporting period has been to extend the understanding of the factors influencing the generation of radon from the ground and to determine the relationship with house levels. The two counties Somerset and Derbyshire, were chosen for the study.

Somerset adjoins the designated radon affected counties of Cornwall and Devon, and a large NRPB housing data set is available. Rock types ranging from various limestones through calcareous shales to clays, and relatively unconsolidated sandstones to competent sandstones and shales, are available for testing. The rocks are characterised by a small range of uranium concentrations but a wide spread of bed-rock permeabilities. This contrasts with the other areas surveyed under the programme where the uranium concentrations were more widespread. Somerset was south of the limit of ice during the Pleistocene and retains many of the erosional features which predated this period. Head, formed under periglacial conditions, mantles much of the area, but is absent or sparse on limestone terrains. It is an unstratified or poorly stratified accumulation of rock fragments of local origin, mantling high ground but often transported by solifluction onto valley slopes and bottoms. Another common overburden is valley terrace deposits. These line river valleys at a higher level than the current flood plain. Since the area has an extensive coast-line there are also thick deposits of estuarine alluvium in low lying coastal areas which extend inland. Estuarine alluvium is clay dominated and relatively impermeable.

There are also thick deposits of peat underlying large areas of low ground. Overlying the Cretaceous Chalk there is commonly a residual overburden deposit: Clay-with-Flints. This is of varying age but is characteristically much more uraniferous than the parent chalk.

The field work took place during September 1992. Soil gas surveys and geological data have been compared with the NRPB house data set. By considering the permeabilities and the radon in soil gas values a reasonable relationship exists with the percentage of houses above the action level. This relationship enables the geochemical data to aid the identification of areas of high radon potential where house data are absent or sparse, and to advise on the construction of contours for maps based upon house data.

Considerations of the aquifer characteristics of the bed-rock give an indication of the permeability to fluids passing through the bed-rock and hence give an indication of the likely transport of ground gas. There are many aquifers, both exploited and potential, in the area and consideration, in any modelling exercise, must be given to the characteristics of these. The properties may be summarised:

> Granular aquifers. Primary porosity, fairly homogenous, isotropic, Darcy's Law applies, flow is slow and laminar.
>
> Fractured rock aquifers. Secondary permeability along joints, bedding planes and faults. Less homogenous and isotropic but depends on scale. Darcy's law sometimes applies, flow may be turbulent.
>
> Karst aquifers. Ternary permeability by solutional enlargement of fractures. Inhomogeneous, anisotropic, Darcy's law does not apply. Flow is irregular and almost always turbulent.

Generally the aquifers are separated from each other by aquicludes. The main aquicludes are clay bearing formations, although some sandstones have properties which similarly prevent the rapid flow of fluids.

The main granular aquifers are the sands such as the Upper Lias Sand Formations. Fractured rock aquifers are typified by the harder sandstones such as those in the Coal Measures or some of the least karstified limestones.

Karstic Aquifers are represented by the major limestone units. The Carboniferous Limestones in particular have extensive cave systems, whilst many of the other limestones show evidence for small scale karstic and epi-karstic development.

It is clear that there are some rocks which show characteristics common to several aquifer types. The Lower Cretaceous Chalk deposit for example is a highly porous rock but the necks of the pore spaces are so small that there is little transfer of fluids between the pores. Because of the slow flow of water in this regime the main migration route for radon in the bulk of the rock is by diffusion, but the distances in water are relatively small, being limited to about 10 cms. There is thus little transport of radon in the granular regime. The Chalk is however an excellent aquifer because it is mainly a fractured rock aquifer. The radon content of the groundwater therefore reflects the radon emanating from the walls of the fractures. The fracturing is irregular and tends to occur in zones. Some of these fractures are enlarged by karstic processes. Radon in overburden gases reflects this

inhomogeneity, with a wide spread of values reflecting the variable nature of the fracturing.

Some of the geological formations are typified by mixed rock types. For example the Lower Lias ranges in composition from a fairly pure limestone near the northern limit through to dominantly clay with interbedded limestone in the south of the county. An element of judgement is thus necessary in assigning a radon potential to areas underlain by this rock type.

Where there are sufficient houses (>80) house radon distribution values for geological formations in general approximate to the lognormal rule and identify unimodal populations. There is a good relationship between the actual percentage of houses above the Action Level and the percentage expected from the distribution data. A small number of formations show bimodality. The Yeovil Sand Formation (in the Upper Lias) for example, can be divided into two lognormally distributed populations with means of 25 Bq/m^3, accounting for 90% of the data, whilst the remainder has a mean of 125 Bq/m^3. The only other formation with strong evidence for bimodality is the Morte Slates within the Devonian. The data can be resolved into two components the upper 5% having a mean of 370 Bq/m^3, with over 99% of this sub-population being above the action level, whilst the remainder has a mean value of 40 Bq/m^3 and with 0.5% expected to be above the action level.

A comparison between the geological assessment of the individual rock types with the actual data obtained from the housing survey is given in the table. Because the individual housing data are confidential it is only possible to assign a rock type to the data in general terms, and houses are not included in such a data set if the positioning is ambiguous. A small inbuilt bias is thus introduced since houses positioned near the junctions between rock formations are thus under-represented. The classes used relate to: High, where 10% or more of houses are above the Action Level. Moderate, where 1%-10% are above the action level. Low+ where it is judged that the potential is low but there may be isolated high values. Low, where <1% are affected. Agreement is generally good despite the small populations available for certain rock types. The greatest disagreement is with the Cornbrash, which is a clay rich ferruginous limestone. Moderate levels of radon were observed over it and the nature of the rock type indicates moderate permeability. However the housing data indicates that less than 1% is affected.

The county of Derbyshire, which is being surveyed this summer, has contrasting uranium levels in the bed-rock and also contrasting permeabilities. The housing stock for the whole of the county of Derbyshire is being investigated by the NRPB. The logistics of extending out from the well surveyed Chapel en le Frith area, mapped in the first round, to include similar lithologies in neighbouring areas and hence to develop procedures for covering larger areas more efficiently, are being studied. The field data are being collected currently.

Publications:

T.K.Ball, Cameron D.G. and Colman T.B 1992, Aspects of Radon Potential Mapping in Britain. Proceedings of the Fifth International Symposium on the Natural Radiation Environment. Radiation Protection Dosimetry. vol. 25. 211-214.

T.K.Ball and J.C.H. Miles 1993. Geological/geochemical factors affecting the radon concentrations in homes in Cornwall and Devon. Environmental Geochemistry and Health. vol. 15. 27-36

Head of project 6: L M Hubbard

II. Objectives for the reporting period:

Our research has focused on the collection of time series data characterizing radon movement indoors during different seasons and weather conditions. This data has been used in conjunction with physical modelling of the time variation of radon indoors. During the past year our objectives have been to complement our data collection in one test home to include parameters characterizing the movement of radon in the soil gas and entry indoors. With these measurements we plan to determine the component of indoor radon which comes from diffusion from the soil surface into the crawlspace of the house versus the component from pressure driven flow, and how these factors vary during different seasons and weather conditions. These types of measurements are rare in typical living conditions and buildings. Another objective has been to start a collaboration with another CEC contractor in our subgroup, CSTC from Belgium. The collaboration involves the evaluation of the flow of soil gas into the SSI research house using a model in use at CSTC and data from the SSI research house.

The measurements performed at House 902 will continue until the summer of 1994. The data will be used both in statistical analysis of the parameters affecting radon entry and movement indoors and in physical modelling of these processes. The statistical and physical modelling efforts will be emphasized in the next year. The statistical analysis will be performed using the BMDP statistical package available at our institute. With regards to the modelling of radon indoors, we plan to begin a collaboration with researchers at the Swedish Building Research Institute in Gävle. Their standard model of multizone indoor airflows will be used for calculation of airflows in our existing radon flow model. The cooperation with RISOE in Denmark on the study of radon entry using the soil transport model from RISOE and data from the SSI research house will be initiated, with a visit of Claus Andersen from RISOE, Denmark, to SSI, Sweden, in October, 1993. The collaboration with CSTC on the evaluation of the flow of soil gas into the SSI research house will continue.

III. Progress achieved including publications.

The ongoing motivation for our research is to obtain sufficient understanding of the mechanisms driving radon dynamics in buildings to be able to simplify the modelling and prediction of indoor radon concentrations and prescribe adequate mitigation at a minimum cost to the household energy consumption. To this end, the following progress has been made during the past year. Time series data collection has progressed at SSI research house 902 during all four seasons of the year 1992/1993. The third year of continuous data collection at house 902 will be completed in October, 1993. The weather parameters wind speed, wind direction, and barometric pressure were added to the continuously collected data set in the fall of 1992. During one week in July and another week in August, 1993, the house was vacant and windows and crawlspace closed in the same way as during the winter data collection. This allows comparison of the house dynamics in the summer versus winter weather conditions with the house in the same structural configuration.

In addition, the following equipment has been fabricated with the goal of measuring both the pressure driven flow and diffusion components of radon entry . Three soil radon detectors based on digital counters which count alpha-decays from radon and progeny have been built and calibrated. Housing to hold the counters in the soil have been constructed. The computer dedicated to the time series data collection has been reconfigured and the software expanded for collection of up to four digital signals.

The three digital counters are currently recording continuous time series measurements of the variation of radon in the soil gas in three different locations at the SSI research house. All three counters are buried 1 meter deep in the soil. Two of the counters are located 1 meter from the perimeter of the house centred on two different sides, north and east. The third counter is buried 1 meter deep directly under the centre of the house. The readings from the three locations differ by a factor of two, with the north side varying around 43,000 Bq/m^3, the east side varying around 20,000 Bq/m^3, and under the house varying around 32,000 Bq/m^3.

With continuous radon measurements in these locations, we should be able to observe changes in the soil gas radon concentrations due to the effects of the weather versus the effect of the house on the soil. The house affects the soil through the stack induced pressure gradient between the house and the soil, and through pressure differences due to the wind. Also, the soil under the house remains dry, so that the flow properties of the soil, i.e., the permeability, which changes with changing water content, remains constant under the house but does not outside the perimeter. How much these differences affect the time variations, or dynamics, of the soil gas radon content will be one interesting observation of these measurements. The degree to which these dynamics change with seasons will be another. So far we have observed only the summer data, which shows some interesting variations of the radon in the soil on the north side of the house with the wind and barometric pressure.

During the period of time that the floor of the research house was opened up to install the radon counter, in-situ flux measurements were taken of radon gas emanation from the surface of the soil in the crawlspace. A series of measurements were taken over five days in July, 1993. The weather conditions were excellent for these measurements. There was no wind, and the outdoor, indoor, and crawlspace temperatures were as close to equal as weather at the research house ever permits. Thus the stack induced pressures were at a minimum or nonexistent during the flux measurements.

The measurements were performed using an accumulator placed on the surface of the ground. Radon emanating from the ground was allowed to build up in the accumulator until equilibrium was reached. A continuous radon monitor was attached to the accumulator and the data digitally recorded. Our initial evaluation of the data gives a summertime flux of radon from the crawlspace soil equal to 0.2 Bq/m^2sec. We plan to compare this source of radon to the crawlspace with the source from pressure driven flow, initially comparing the two as a function of seasonal changes in the weather parameters.

Progress Report

Contract: **FI3P - CT930074** **Sector: C12**

Title: **Evaluation of the combined helium/radon in soil gas mapping methodology as an indicator of areas in which elevated indoor radon concentrations may be found.**

1)	Colgan	RPII
2)	O'Connor	Geological Survey (Irish)
3)	Van den Boom	ENMOTEC GmbH
4)	Porstendörfer	Univ. Göttingen

1. Summary of Project Global Objectives and Achievements

Radon monitoring in Ireland to date has indicated that the majority of the elevated indoor radon concentrations are spatially associated with limestone regions, which comprise up to 40% of the land area of the country.

Previous investigations in a limestone region in western Ireland (contract Bi7 - 0059) have shown that this rock type is prone to dissolution by percolating groundwater (karstification) and that the consequent increase in permeability is probably significant in facilitating the migration of free radon to the surface. Consequently built up areas underlain by such limestone which have not been extensively surveyed for indoor radon concentrations may also have a significant proportion of houses with elevated indoor radon levels.

Radon mapping methodologies, based on established soil-gas mapping techniques are being developed in several EC and non-EC countries. The ability of these mapping procedures to successfully identify areas with elevated indoor radon concentrations has not so far been critically assessed. This project aims, for the first time to assess the effectiveness of the combined Helium/Radon in soil-gas mapping methodology in identifying such areas.

The global objectives of the project can be summarised as follows:

a. To assess the effectiveness of the Helium/Radon in soil-gas mapping methodology as an indicator of areas in which elevated indoor radon concentrations may be found.

b. To investigate the temporal and spatial patterns of indoor radon concentrations in houses.

c. To investigate the influence of meteorological parameters on the transport of radon from soil air into indoor and outdoor air.

d. To improve our understanding of the geological factors which control the generation, migration and ingress of radon into houses.

The first stage of the project is now completed with the execution of an integrated field campaign by the project team in the carboniferous limestone regions of Claremorris, Co. Mayo and Milltown, Co. Galway during the period May 10th - 23rd 1993.

A total of 1244 alpha track (Cr-39) passive radon detectors have been issued to participating householders in the survey area and results are expected in January - February 1994.

A sampling programme of 1018 measurements incorporating 526 soil gas radon determinations, 179 soil-gas helium determinations and 313 permeability measurements has been completed and preliminary analysis of the data suggests that the selected area of Claremorris can be classified as an area with high radon potential and high risk for elevated indoor radon concentrations.

Reconnaissance mapping of the bedrock geology and glacial geology of the survey areas has also been completed as a background to the soil gas radon and helium surveys.

At a preselected house in Milltown, Co. Galway the transport of radon from soil air into the indoor and outdoor air was investigated. During the field campaign a programme of continuous monitoring of

a. soil gas radon concentration at 1m depth

b. subslab radon concentration

c. indoor radon concentration at several locations inside the house

d. pressure differences between indoor air, subslab air and outdoor air and

e. temperature differences between indoor air and outdoor air

was carried out.

A ground-probing radar survey of this house site in addition to the site of a local school was also undertaken to characterise surface conditions and help delineate possible radon migratory routes into the buildings.

Results to date indicate that radon entry into the house is governed by the pressure difference between soil and indoor air, thus confirming a strong convective flow of radon. During the measurement period the pressure difference between soil and indoor air was mainly governed by the wind pressure applied to the house. Reducing the pressure difference between soil air and indoor air by about 1Pa by blowing attic air into the ground floor of the house reduced the indoor radon concentration by a factor of 13.

Preliminary results from the ground probing radar surveys suggest that a geological fault, contact or lineament runs beneath the house at Milltown, which could possibly represent a pathway for radon movement to the surface. At the school test site severe attenuation of the radar signal is evident in the immediate vicinity of the school building. Such attenuation of radar signals is normally encountered over low resistivity clays and subsequent resistivity surveys at the site suggest that the school may have been constructed on a topographic depression filled with clay.

During construction some of this clay was excavated and the depression infilled with more permeable hardcore, thus presumably facilitating easy movement of radon to the school substructure. Further processing of the GPR data is currently underway.

During the remaining period of the project all field data will be evaluated and interpreted. All available geodata for the survey area will be computer integrated and relevant maps produced and a final report prepared to the end of May 1994.

I. Head of Project: Dr. Colgan

II. Objectives for the Reporting Period

a) To approach and solicit on behalf of the research group the participation of householders in the project and to determine the geographical distribution of indoor radon concentrations in the Claremorris District Electoral Division (DED).

b) To locate a vacant house within the general survey area with elevated indoor radon levels, and obtain permission for the use of this house in subsequent detailed follow-up investigations by the research group.

c) To conduct a site investigation at the chosen house. This investigation would include a ground probing radon (GPR) survey of the site in addition to a GPR survey of the interior of the house. In conjunction with our partners continuous sub-slab radon measurements would be carried out on site.

d) To collect soil samples for natural radioelement analysis by high resolution gamma-ray spectrometry from locations geographically distributed throughout the survey area.

III. Progress Achieved including publications

Eight hundred and eighty two householders in the Claremorris DED were contacted by the RPII regarding participation in the project. Following a publicity campaign involving local media and public meetings 250 householders agreed to take part. In July 1993 500 alpha track (Cr-39) passive radon detectors were issued to the participating householders, for a minimum exposure period of 6 months. Thirty one houses geographically distributed throughout the survey area were selected for detailed investigation of the temporal and spatial pattern of indoor radon levels, and in August 1993 744 passive radon detectors were issued to this select group. These detectors will be retrieved at 1 month intervals for processing.

During April 1993 investigations were carried out in the survey area to locate a vacant house with elevated indoor radon levels, and also obtain permission of the owner for the use of the house during the field campaign.

Following discussions with local auctioneers and estate agents 9 potential houses were monitored using the electret-passive environmental radon monitor (E-Perm) system. Short-term (6-13 day) average radon concentrations ranged from 24 Bqm^{-3} up to 143 Bqm^{-3}, but none fitted the necessary criteria. As a fall back position the RPII contacted the owner of a house, some 15 kilometres away from the survey area, which was known to contain elevated indoor radon concentrations, and permission was granted for use of the house. Short-term (15 days) average radon concentrations throughout this house ranged from 306 Bqm^{-3} up to 428 Bqm^{-3}.

Field work commenced in May 1993. The house locations of all participating householders were plotted on 1:2500 scale O.S. maps. Ground probing radon surveys were carried out at the house chosen for detailed investigation and at a local school which in recent years, prior to mitigation, had radon concentrations in excess of 1000 Bqm^{-3} in several classrooms.

At two location in the house holes were drilled through the slab to facilitate the active measurement of sub-slab radon concentrations. These measurements were jointly carried out by the RPII and Göttingen University. The RPII used a Pylon AB-5 Radiation (Lucas Cell) System to complete these measurements.

Soil samples from 5-30cm depth were collected at 44 locations throughout the survey area, for analysis by high resolution gamma ray spectrometry.

Publications

Porstdendörfer, J., Butterweck, G., Madden, J and Reineking, A. "Influence of meteorological parameters on the transport of radon from soil air into indoor and outdoor air". Presented at the First International Workshop on Indoor Radon Remedial Action, Rimini, Italy, 27th June to 2nd July, 1993.

IV. Objectives for the next reporting period

During the remaining time period of the project all track etch detectors will be recovered and processed, and occupancy patterns for residents in the survey area evaluated.

The GPR survey data will be processed and analysed in greater detail, and all available geodata for the study region will be computer integrated and relevant maps produced. The indoor radon data together with the site specific data will be assessed in the context of the geological, geophysical and Rn/He mapping.

Head of project 2: Dr. O'Connor

II. Objectives for the reporting period

(a) Provision of topographic maps (1:10,560 and 1:2,500 scales) of survey areas to partners RPII and ENMOTEC and assistance in the planning of field surveys by the project team.

(b) Systematic geological reconnaissance of selected survey areas including the collection of representative rock and soil samples for radioelement abundance analysis.

III. Progress achieved including publications

(a) Participation in project co-ordination meeting at RPII, Dublin (8th Feb. 1993) to select survey areas and plan field campaign.

(b) Provision of topographic maps of selected survey areas to partners RPII and Enmotec.

(c) Participation in field survey campaign 10 - 23 May, 1993 in Claremorris area (Co. Mayo) and Miltown area (Co. Galway).

(i) Reconnaissance mapping of bedrock geology and glacial geology of both survey areas completed as a background to soil - gas radon and helium surveys.

(ii) Collection of a suite of representative rock and soil samples completed for survey areas (with RPII).

(iii) Assistance in the preparation of two detailed site plans with RPII as a basis for investigating local correlation of indoor radon concentrations with local geological parameters.

(iv) Preliminary evaluation of correlations between geology and soil-gas radon concentrations carried out.

Publication

O'Connor, P.J. (in press) correlation of indoor Rn with geology. Contribution to Radon Research Notes, U.S. Dept. of Energy.

IV Objectives for the next Reporting period

(a) Preparation of final geological maps of survey areas, incorporating all extant geodata in GSI files and final field checks.

(b) Assessment of radioelement contents of rock and soil samples (with RPII) in relation to measured Rn concentrations.

(c) Evaluation of correlations between geology, soil-gas Rn concentrations and indoor Rn concentrations (with RPII and Enmo tec).

(d) Preparation of progress report to end May 1994.

ENMOTEC GmbH
Bismarckstr. 80
72072 Tübingen
Germany

EC-Project No. : FI3P - CT93 - 0074
Project-Title : Evaluation of the combined Helium/Radon in Soil Gas Mapping Methodology as an Indicator of Areas in which Elevated Indoor Radon Concentrations may be found
Project Manager:

First Progress-Report

1. Objectives of the Reporting Period.

Four main objectives were given for the investigation in the area of Claremorris (West Ireland):

- Application of a tested helium-radon method in soil gas geochemistry
- Measurement and determination of soil permeability and establishment of the regional radon availability
- Regional radon and helium distribution in soil air in an area of 4x4 km surrounding the village of Claremorris
- Radon concentration in soil gas samples in the direct vicinity of the Convent Primary School in Claremorris and a selected house in Milltown (Co.Galway) (Cooperation with University of Göttingen).

2. Objectives for the next period (until May, 1994)

The following objectives for the next period are:

- Detailed evaluation and interpretation of the regional survey data
- Evaluation of the data obtained in the vicinity of the selected buildings
- Evaluation of the traverse data
- Determination of the radon availability
- Regional distribution of helium and radon concentrations in soil air
- Evaluation of repeater data in comparison with meteorological parameters
- Attempt to establish a correction equation to obtain corrected field data
- Submission of maps, plans and profiles.

3. Progress achieved including publications

3.1. Project Preparation

Preparation for the project started two months before fieldwork commenced in Ireland. The necessary equipment was checked, field-tested and improvements made where necessary. In cooperation with the University of Göttingen (Prof. Porstendörfer) quality and efficiency of Lucas cells were controlled and tested.

3.2. Field work

Field work has been carried out in May 1993 in an area 4x4 km including the town of Claremorris in Western Ireland (see figure 4). Three technicians were involved in the sampling campaign, two scientist worked in the field laboratory and one scientist of the participating U.S. Geological Survey in Denver worked as well in the field as in the laboratory. Soil gas samples were collected from 1 m depth near roads and tracks in the area of investigation. Distances between sample stations were approximately 200 - 250 m. The regional study included radon and helium sampling and determination of soil "permeability" at almost every soil gas sampling point. Detailed sampling for radon and helium was carried out around the Convent Primary School in Claremorris where high indoor radon levels were measured by R.P.I.I. (Dublin) in recent years. A selected house in Milltown where indoor measurements were carried out by the University of Göttingen simultaneously was surrounded by soil gas sampling stations for the determination of radon and helium concentrations in soil air.

Because of the generally high levels of radon concentration in the collected soil gas samples in the studied area it was necessary to sample a traverse of approximately 25 km length to determine the extension of the radon elevated area of Claremorris. This traverse was sampled between Claremorris and Castlebar.

During the field-work period the following soil gas samples were collected:

- **Regional survey Claremorris**

Radon samples	250
Helium samples	150
Permeability measurements	250

- **Convent Primary School**

Radon samples	39
Helium samples	17
Permeability measurements	39

-	**Milltown House**	
	Radon samples	24
	Helium samples	12
	Permeability measurements	24
-	**Traverse Claremorris-Castlebar**	
	Radon samples	39
-	**Radon-Repeater**	
	Radon samples	174
Total Sampling Programme		**979**

3.3 Laboratory work

All samples collected during daytime were analyzed the same day. This was especially necessary for the radon samples which have a half-life time of 3,8 days. On the other hand immediate results made decisions for the next day sampling programme easier and more effective.

Soil gas samples taken in the region of Claremorris were treated statistically Preliminary results can be shown here:

Table 1: Statistical parameters of the helium and radon concentrations in soil gas sample in the area of Claremorris (West Ireland)

	Helium	**Radon**
Sample size	150	250
Median	5268 ppb	67240 Bq/m3
Variance	9249,5	
Standard Deviation	96,2	59600
Minimum value	5094 ppb	0
Maximum value	5624 ppb	392700 Bq/m3
Range	530	
Lower quartile	5247 ppb	35200 Bq/m3
Upper quartile	5329 ppb	94600 Bq/m3

Frequency histograms are shown in figure 1 (helium) and 2 (radon).

Helium values are strongly elevated and the radon concentrations are very high. A first evaluation of the data show that the selected area of Claremorris can be classified as an area with high radon potential and high risk for elevated indoor radon concentration.

Further results obtained in the detailed study in the vicinity of the Convent Primary School in Claremorris have been published at a poster session during the EC - Meeting in Rimini June 27 - July 2, 1993.

The results are summarized in figure 3 of this report.

List of figures

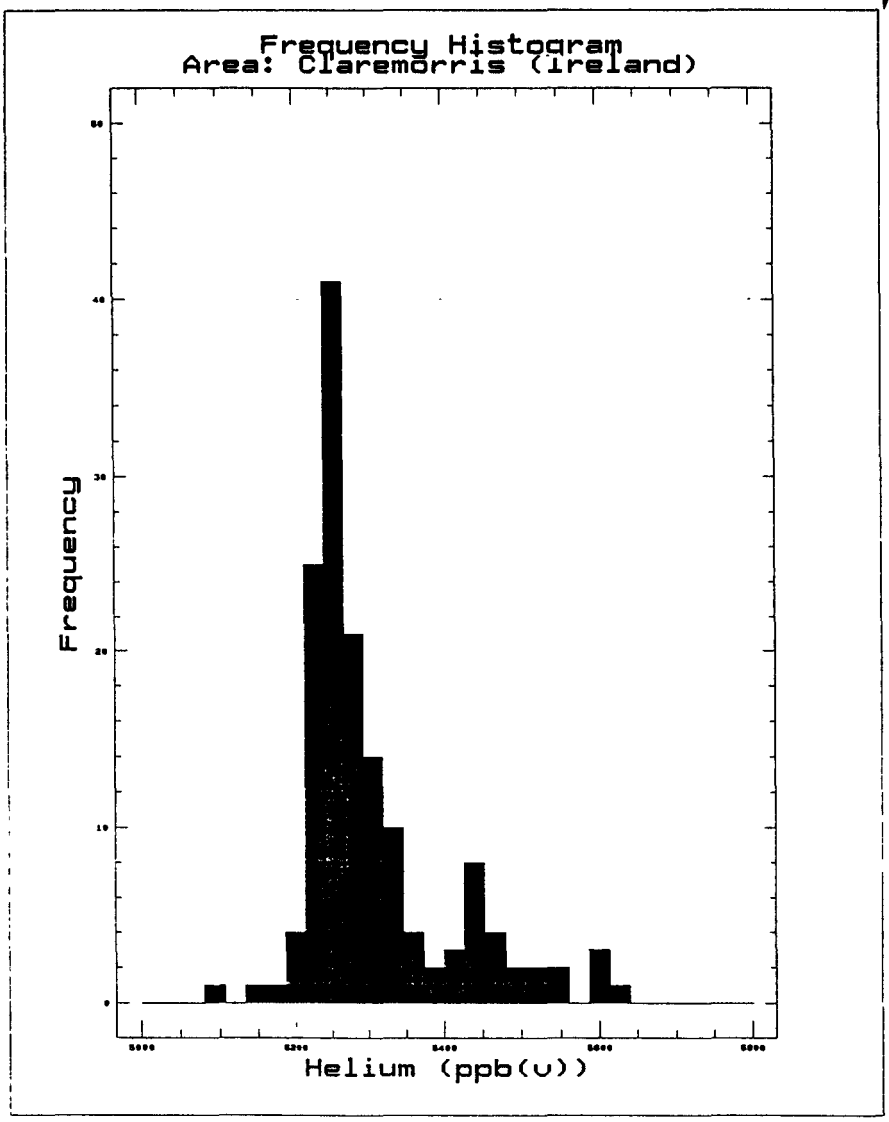


Fig. 1: Frequency Histogramme

150 Helium samples

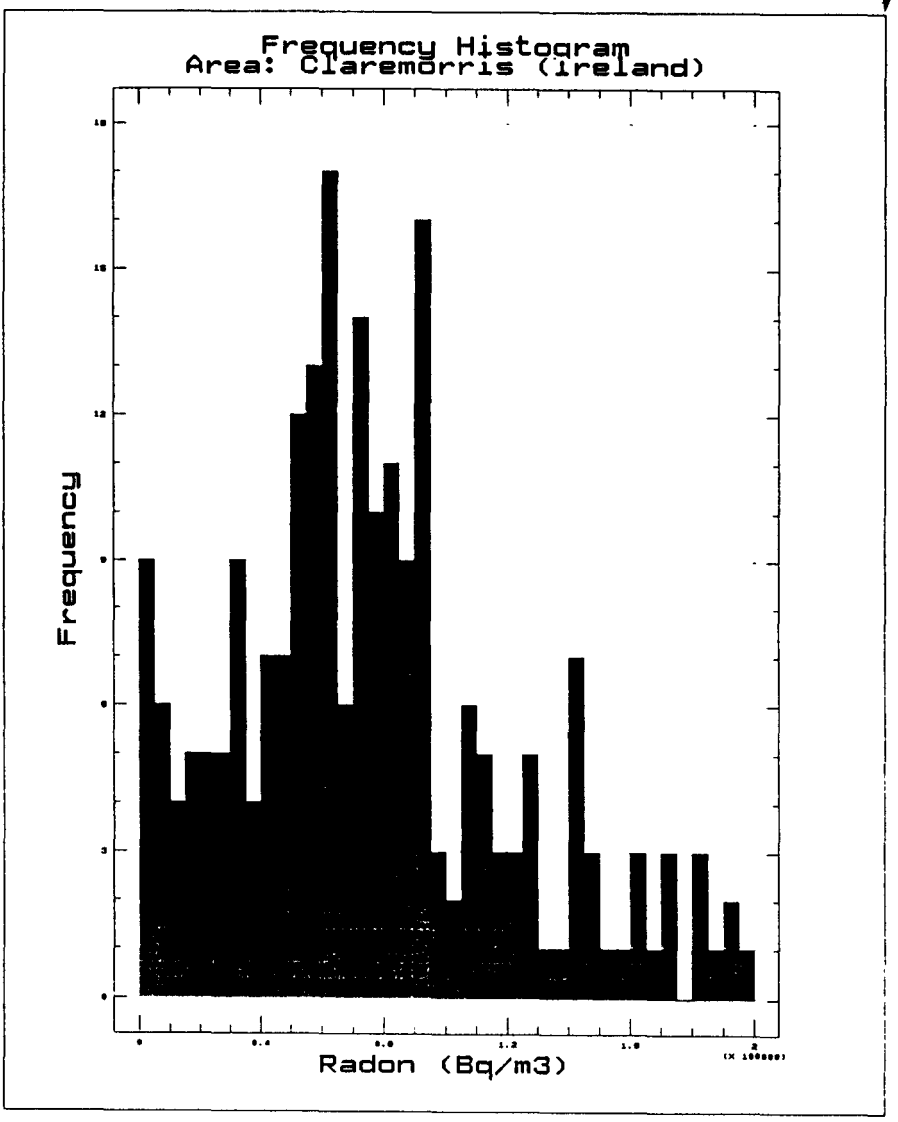


Fig. 2: Frequency Histogramme

250 Radon samples

Fig. 3: Regional Distribution of Radon in Soil Gas

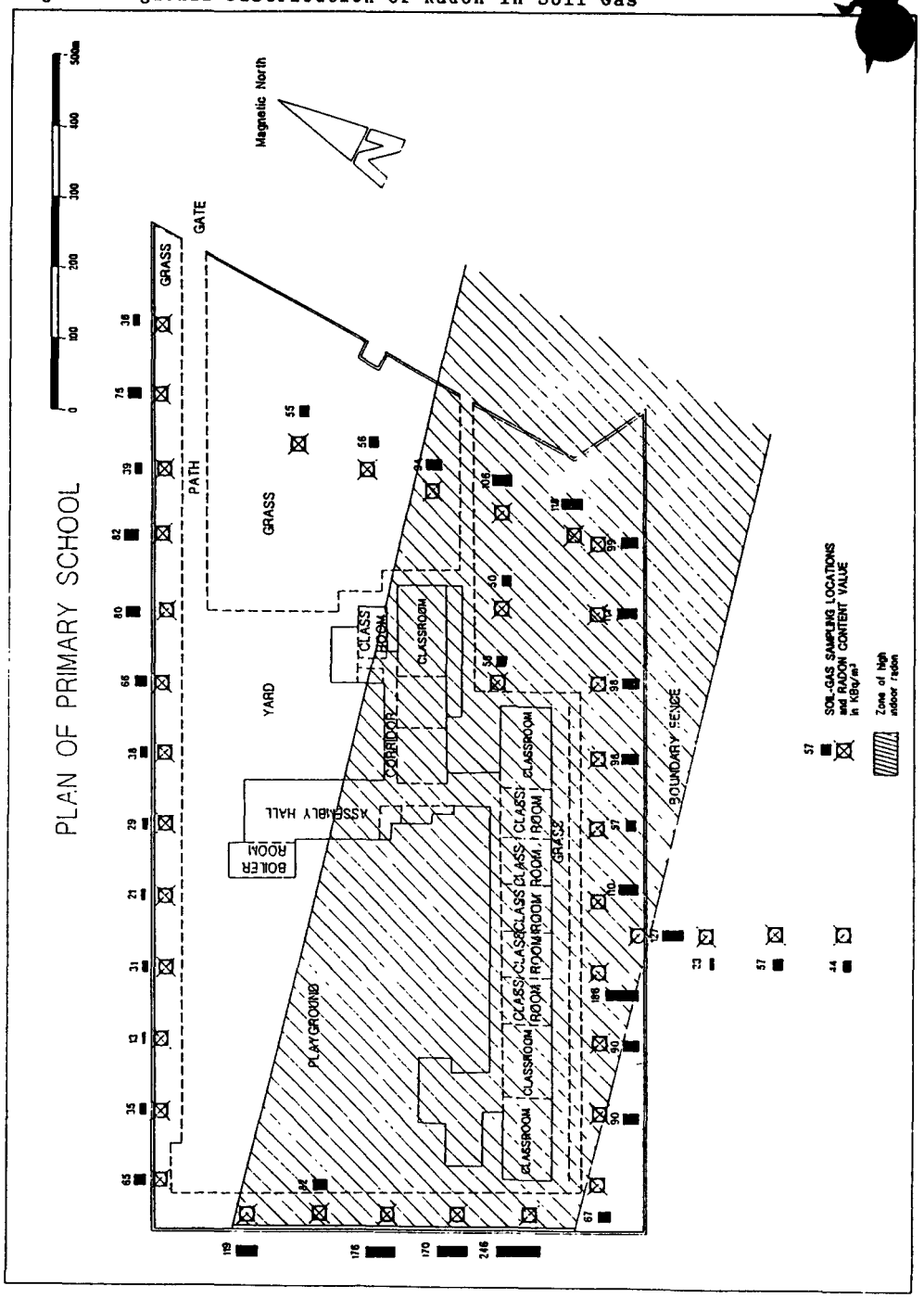
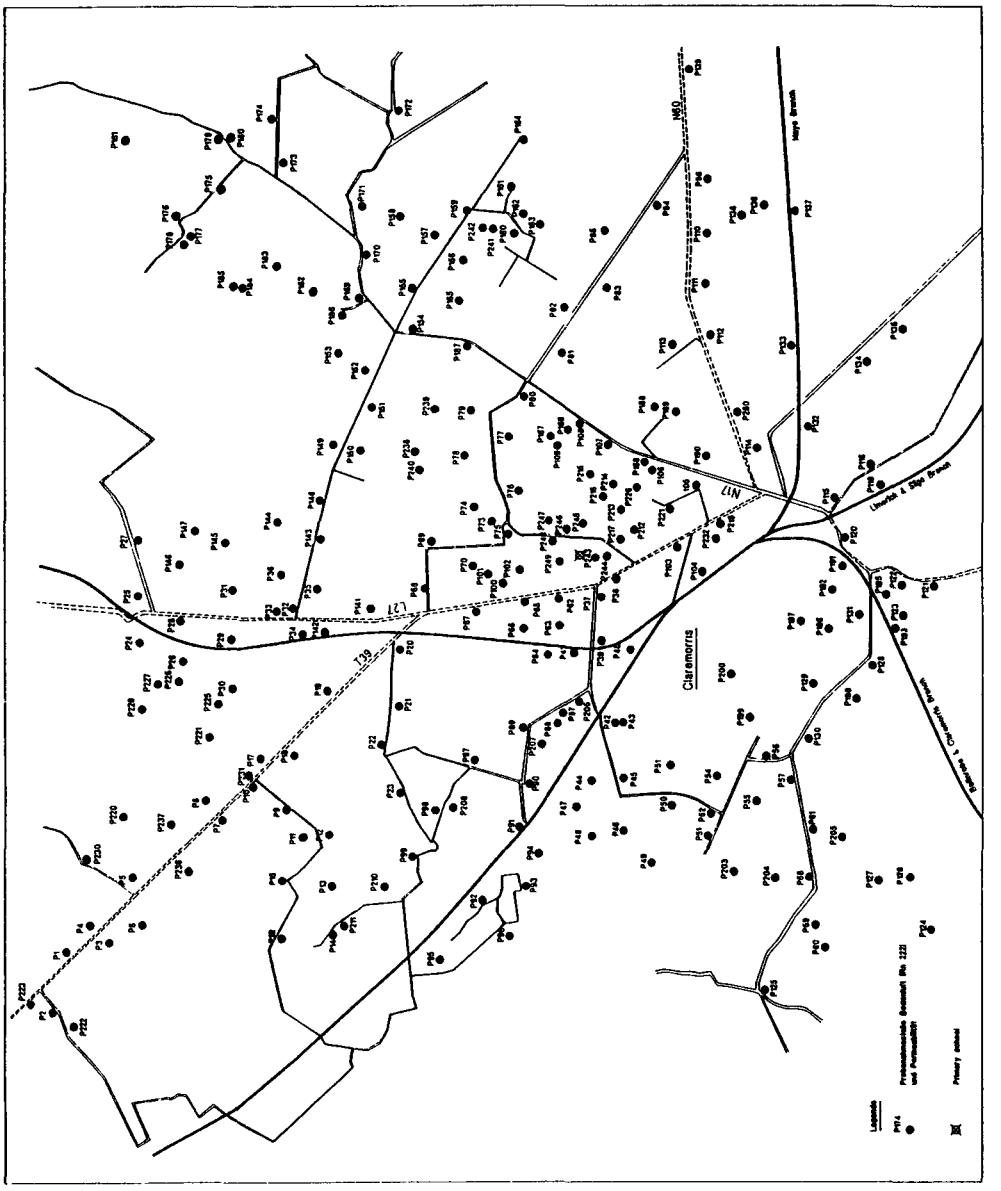


Fig. 4: Location Map of soil gas samples in the Area of Claremorris (West Ireland)



Head of project 4: Dr. Porstendörfer

II. Objectives for the reporting period

During the reporting period a measuring campaign of all in the project included groups in Ireland was planned, where the IL should take the part looking upon the change of radon concentration in soil and indoor air due to meteorological conditions. Therefore a continuously monitoring of

- soil radon concentration in 1 m depth (sampling depth of the Enmotec group)
- radon concentration beneath the slab of a uninhabited measuring house
- indoor radon in several parts of the house
- pressure differences between indoor air, subslab and outdoor air
- temperature differences between indoor and outdoor air

was planned.

III. Progress achieved including publications

The transport of radon from soil air into houses was investigated at a building in Ireland. It could be shown, that radon entry in the house is governed by the pressure difference between soil and indoor air, thus confirming a strong convective flow of radon. The pressure difference between soil and indoor air is strongly dependent wind speed. Subslab radon concentration shows a similar variation as the soil radon concentration in uncovered soil, just smoothed, due to a compartment structure below the slab. Reducing the pressure difference between soil air and indoor air about one Pascal by blowing attic air into the ground floor of the building yields about a factor of 13 reduced indoor radon concentration.
Results have been presented at the 1. Int. Workshop on Indoor Radon Remedial Action, Rimini, Italy, 27 June-2 July

IV. Objectives for the next reporting period

Final evaluation of the data measured in the measuring campaign in Ireland from May 10th to May 20th 1993.
Construction of a probe for the simultaneous and continuous measurement of radon and soil permeability in four variable steps between soil surface and 5 m depth. The probe will also be used to measure the vertical pressure distribution in the soil.
Building and calibration of specially for the measurement of soil radon and thoron activities designed radon chambers (method: electroprecipitation).
First test measurements in with the new probe and radon chambers in the vicinity of Göttingen.

Achievements in the reporting period:

During the measuring campaign in Milltown (Ireland) radon concentrations in the ground floor (all rooms open), attic, 50 cm below the slab, outdoors and in the soil (1 m depth) and additional the temperatures in the ground floor, in the attic and outdoors. The pressure differences between outdoor atmosphere, attic and subslab soil to the indoor air were measured. Additional meteorological data, like wind speed, atmospheric pressure, soil temperatures and rain intensity were supplied by the Meteorological Service of Ireland (Claremorris station, 10 km from Milltown). The measurements were performed during a joint measurement together with the Radiation Protection Institute of Ireland and ENMOTEC Company, Germany during the time between may 10th and may 20th. The house was built 20 years ago in a plane region. The subslab zone is divided by footings into compartments of the size of the overlying rooms.

The radon and thoron activity concentration were measured continuously with the method of electroprecipitation of freshly in a decay volume produced daughter products on a surface barrier detector and alpha-spectroscopy. For room air measurements a 5 l chamber was used with a detection limit of 6 Bq/m^3 for radon and 12 Bq/m^3 for thoron (1 hour counting interval, 1 σ statistical uncertainty). The soil air measurements were carried out with a chamber volume of 0.27 l and detection limits of 60 Bq/m^3 and 150 Bq/m^3 for radon and thoron respectively.

The probe for the soil radon measurements consists of an steel pipe (diameter 1 cm) containing two stainless steel tubes of 1 mm inner diameter, sealed against the hull pipe. The sample air was sucked through one tube with a flow rate of 10 l/h, passed the detector and was pumped through the second tube back into the soil, thus minimising the influence of the measurement on the soil radon concentration. For soil measurements the probe was driven into the soil, using the whole length of the hull pipe as sealing against entry of outdoor air. For the measurement of subslab radon the probe was sealed in the drill hole with a standard silicon sealing compound.

Pressure differences were measured with a micromanometer (MKS Baratron) with a resolution of 0.01 Pa and air temperatures were measured with thermistors (resolution 0.1 °C). Ventilation rates were computed from the decrease of artificially elevated CO_2 concentrations.

A dependence of the indoor radon concentration of the pressure gradient was confirmed with measurements performed at Milltown (Ireland). The variation of the indoor radon could be reproduced by a source factor, calculated from pressure gradient between subslab and indoor air and subslab radon concentration, during the whole measuring campaign, confirming that the pressure difference between indoor and subslab air governs the radon entry by convective flow from subslab air into the building (Fig. 1). The pressure difference between subslab air and ground floor is varying according to the pressure difference between outdoor air and ground floor (Fig. 2), aside from a reduction of amplitude by a factor of about 9, which indicates a massive transport resistance between the subslab air and the open atmosphere, probably due to the compartment structure of the subslab region.

Considering the subslab radon concentration in comparison to the soil radon concentration in 1 m depth, a smoothing of the temporal change can be observed (Fig. 3), which is also due to the compartment structure of the subslab section.

During the measuring period in Milltown (Ireland) the pressure difference between outdoor and indoor air and also between subslab and indoor air was mainly governed by the wind pressure applied to the house (Fig. 4). As the house was not heated during the measuring campaign, the influence of the temperature difference between indoor and outdoor air on the

pressure difference (stack effect) had to be weak and could not be separated from the dominating dependence of the pressure gradient on wind speed.

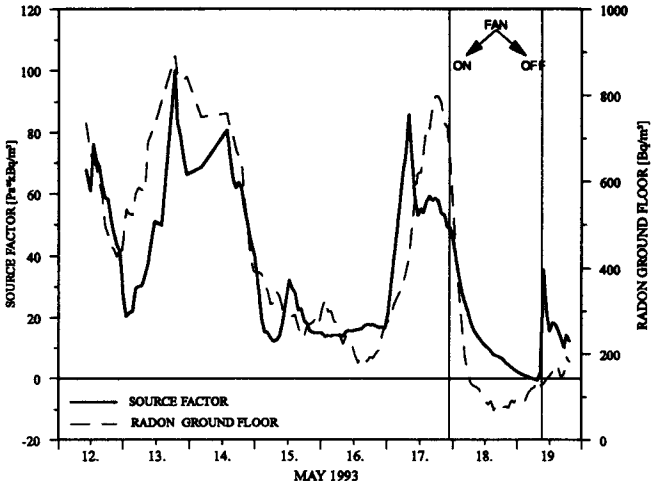


Fig. 1: Comparison of the indoor radon concentration to a source factor (product of pressure difference indoor - subslab and the subslab radon concentration).

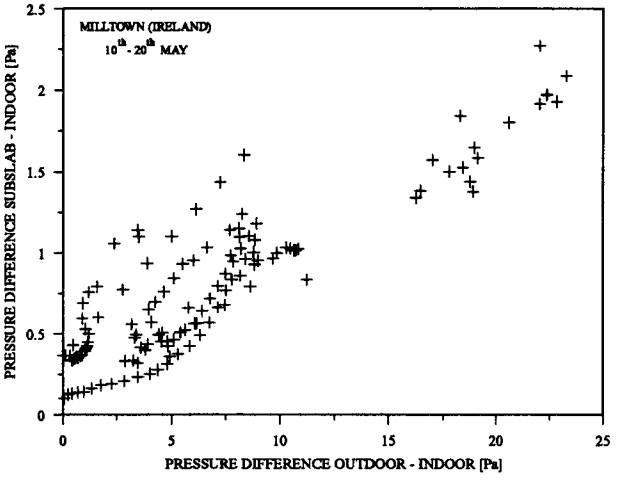


Fig. 2: Dependence of the pressure difference between subslab region and ground floor on the pressure difference between outdoor air and ground floor.

As the radon entry into the building is governed by the pressure difference between subslab and indoor air an experiment was performed to reduce radon entry by an artificial overpressuring of the house with a small fan blowing air from the attic into the ground floor. The experiment started at May 17th and ended 19th (for the influence on source term and radon concentration see Fig. 1). The distribution of the ratio of pressure difference between subslab and indoor air to the wind speed was shifted to lower values. If the pressure

difference between subslab region and indoor air is considered roughly proportional to the wind speed, the ratio between pressure difference and wind speed describes the radon entry largely independent on wind speed and as it is the dominating influence parameter on weather conditions. Under normal conditions the average value was 0.31 Pa s/m (range 0.08 - 2.72 Pa s/m) and with fan blowing attic air into the ground floor 0.04 Pa s/m (range 0 - 0.16 Pa s/m). If the radon concentration in the ground floor is considered proportional to the pressure difference (Fig. 1), the reduction of the average radon concentration in the ground floor due to the effect of the fan would be about a factor of 13. The ventilation rate with operating fan ($V = 0.31\ h^{-1}$) was only slightly increased compared to the normal conditions ($V = 0.24\ h^{-1}$) and its effect on radon reduction was neglected.

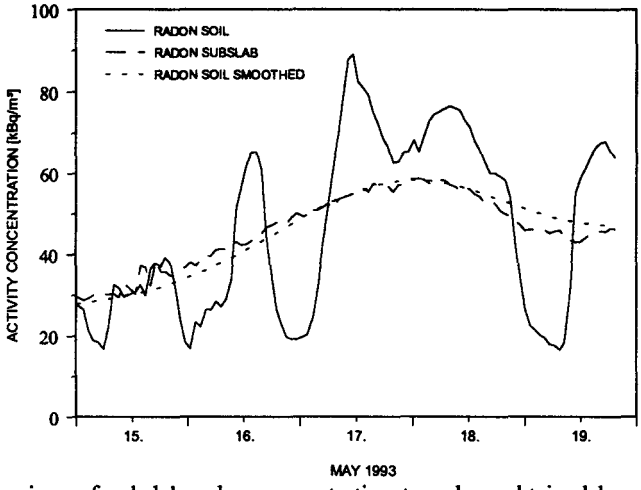


Fig. 3: Comparison of subslab radon concentration to values obtained by smoothing of the undisturbed soil concentration.

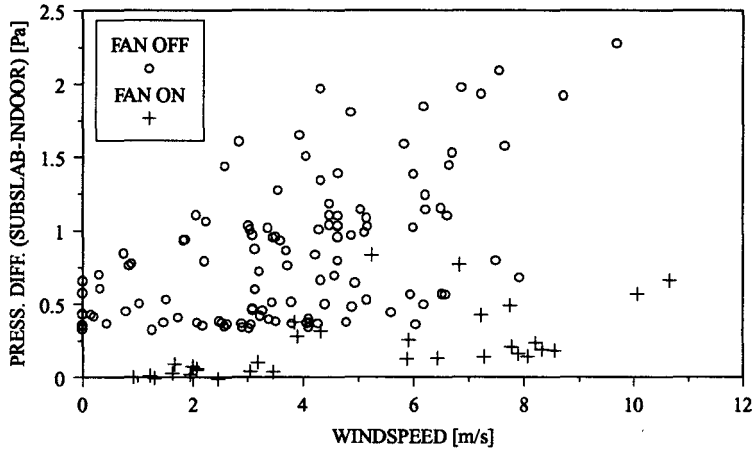


Fig. 4. Dependence of the pressure gradient between outdoor and indoor air on the wind speed under normal conditions and with a fan blowing attic air into the ground floor.

Progress Report

Contract : **F13P-CT920019** **Sector: C13**

Title : Comparative assessment and management of radiological and non-radiological risks associated with energy systems.

1) Dreicer CEPN
2) Friedrich Univ. Stuttgart
3) Uijt de Haag RIVM

I. Summary of Project Global Objectives and Achievements

The global objectives of this project is to develop a methodology for the comparison of radiological and non-radiological risks associated with energy systems. Within this context of this work the risks considered are being limited to the health and environmental domains. Even after narrowing the boundaries of the assessment, it is still difficult to find a truly common ground for direct comparisons to be made between the different fuel cycles as well as between the different types of risks that can be estimated.

The differing scales of time and space that the impacts can potentially span, create difficulties in making direct comparisons. For example, the impacts from radioactive waste disposal are not likely to be seen for tens of thousands of years and the emission of greenhouse gases from fossil fuel cycles has a potential global impact. Should occupational risks be treated differently than public risks. The integration of low probability but high consequence events is difficult especially when the issues of risk aversion are considered.

In many comparative risk assessments risk indicators are transformed into monetary terms. By using a willingness-to-pay approach to quantification of the risks, some of these factors may be taken into account. On drawback is that site-specific information is usually required and therefore it is difficult to work interactively with these type of results to explore the outcome of different choices.

Within this project a methodology of comparative risk analysis is being explored using multi-criteria utility techniques. Applicable risk indicators for human health and the environment are being developed.

The more specific objectives are :

1) Development and improvement of health risk indicators, including non-radioactive substances, for the use in comparative risk analysis;
2) Compilation of environmental risk indicators used in former risk assessment studies;
3) Development of new environmental risk indicators for the comparative risk assessment methodology being developed;
4) Consideration of the influence time and space have on the comparison of the risks;
5) Construction of a framework for the multi-criteria utility analysis using the data available for the coal and nuclear fuel cycle;
6) Development of a methodology for consistent definition of system boundaries to thereby avoiding comparing energy fuel cycles with different boundaries; and
7) Estimating the range of uncertainty associated with the risk estimates used in the multi-criteria analysis.

In order to develop case studies using the data available for France and Germany, the weighting of the risk indicators and criteria used in the multi-criteria utility analysis must be developed for a few test populations.

Head of Project 1: Ms. Dreicer

II. Objective for the reporting period

During this reporting period :

(1) CEPN has continued to estimate the risks from the nuclear fuel cycle within the context of the DGXII External Costs of Fuel Cycles Project (Joule II). Further developments were made in the analysis of agricultural impact pathways, liquid release pathways (into the sea and rivers) and on the assessment of nuclear accidents. These have been considered within the context of time and space.

(2) A feasibility study on the use of multi-criteria utility analysis (MAUA) techniques for comparative analysis was completed. The exercise used a commercially available software (VISA) with the risk data available for health indicators in the coal and nuclear fuel cycles. Many simplifying assumptions were applied, so the comparison is not valid but it has highlighted the advantages and disadvantages of using this technique.

During the next reporting period, CEPN will complete the risk assessment of the fuel fabrication and conversion stages of the nuclear fuel cycle. As the development of environmental indicators continues for non-radioactive contaminants, the development of indicators for low-level and high-level radioactive contamination of the environment will be considered. The development of these indicators will allow for the integration of environmental factors into the MAUA during the completion of this contract period.

The next step in the development of MAUA methodology is to conduct a more realistic comparison of the coal and nuclear fuel cycle. It is not surprising that the feasibility study for MAUA techniques has shown the importance of the weights given to the different risk criteria. It is clear that these weights are very specific to the population involved in the choices to be made. In the next reporting period, CEPN plans to develop one set of weights for the criteria that can be used in the analysis. With this as a base, it is hoped that during the following years it will be possible to develop comparable sets of weights for different population groups in each of the countries involved in this project.

III. Progress achieved including publications

The continuation of the risk assessment of the nuclear fuel cycle has included the implementation of a marine dispersion model and river dispersion model at CEPN. The assessment of the agricultural pathways has been expanded to take into account the longer-lived radionuclides. At present the methodology integrates the doses to a defined population until 100,000 year into the future. The analysis of a large-scale nuclear accident is one of the most important components of the risk assessment for the nuclear fuel cycle. The results of this portion of the assessment will ultimately influence the outcome for the nuclear fuel cycle within a comparative analysis. Originally only one scenario was assessed. A sensitivity analysis has been carried out by altering the order of magnitude for the postulated releases resulting from a core melt at a pressurised water reactor.

The initial work using the MAUA techniques has not yielded results for a proper comparison between nuclear and coal fuel cycles but it has demonstrated the feasibility of using the technique, as well as the advantages and limitations. By choosing only 2 fuel cycle options for the comparison, it was necessary to add additional information on the maximum and minimum risk to create two other "options". Environmental consequences were not included in the feasibility study but it is planned to include them in the future. Health impacts data from the literature review and the External Costs of Fuel Cycle Project were used.

According to the main dimensions considered in this project, a set of criteria was defined (time/space, public workers, accidental/normal). Table 1 illustrates the criteria and the risk indicators considered for the two fuel cycles and includes the simple assumptions for the corresponding weights that were assigned.

Table 1. Weights assigned to the criteria used in the feasibility study.

Workers 50		Short term 33	fatal accidents 50
			non-fatal accidents 50
		Medium Term 33	fatal radiological impacts 50
			non-fatal radiol. impacts 50
		Long term 33	severe hereditary effects 100
		Short term 33	fatal accidents 50
			non-fatal accidents 50
Public 50	Local 50	Medium Term 33	fatal radiological impacts 50
			non-fatal radiol. impacts 50
		Long term 33	severe hereditary effects 100
	Regional 50	Medium Term 33	fatal radiological impacts 50
			non-fatal radiol. impacts 50
		Long term 33	severe hereditary effects 100

For the four options designated (coal, nuclear, minimum coal, and minimum nuclear) it was not possible to directly compare the impact of a severe nuclear accident with the coal fuel cycle because an equivalent does not exist for the coal fuel cycle. To integrate the impacts of a severe nuclear accident into the feasibility study, the results were weighted and added to the normal operation impacts for the nuclear options. It was decided that the publics' aversion to risk would be used in weighting the impacts of an accident. At this time, CEPN is not aware of any studies that have quantified this "risk aversion" factor but in general, risk aversion has been estimated to increase the impact by a factor of 1.2 to 2 for nuclear issues. For the feasibility study, a constant value of 1.2 was taken and the impacts were added to the normal operation data.

In choosing a utility function for scoring the estimated risks (impacts) of the fuel cycle options, a different consideration for risk aversion was also required. It was considered that the population's aversion to risk increases with the increasing magnitude of risk, regardless of the origin of the risk. With this in mind, an evolving risk aversion utility function was chosen. If the risk aversion factor is noted as a, then the shape of the utility function is defined by $0 < a(\text{minimum risk}) < 1$ and $a(\text{maximum risk}) > 1$. This is illustrated in the Figure 1 below.

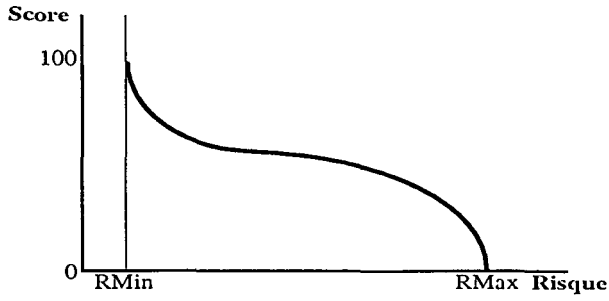
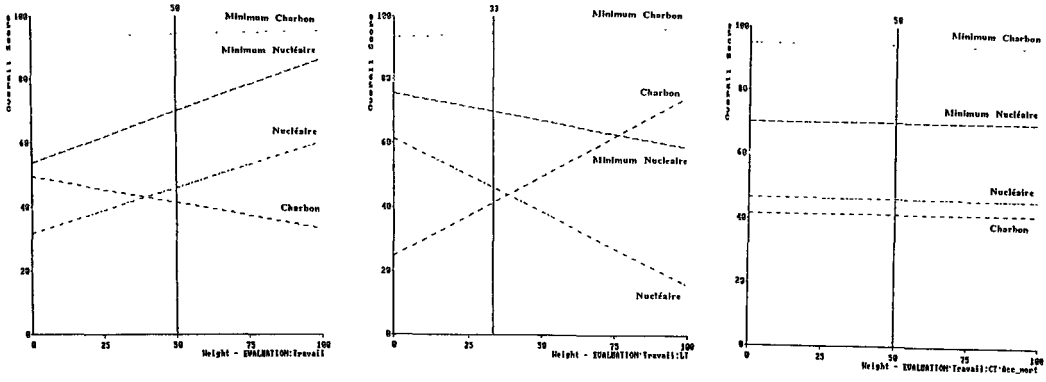


Figure 1. Utility function for an increasing aversion to risk.

Using the data collected, a test case was run to compare the 4 options assuming a utility function as illustrated above. This was then used to test the sensitivity of the system to the variation of the weights assigned to the criteria (Table 1). Figures 2a,b,c illustrate some the preliminary results of the feasibility study for the 4 options, given the simplistic assumptions that were taken.



a b c

Figure 2. Preliminary results of the sensitivity analysis of the feasibility study.

Figure 2 illustrates the results of the overall scoring of the 4 options under different weighting assumptions. The vertical line in the middle indicates when the weights for the occupational/public impacts are both 50%, the ranking of the options is Minimum Coal, Minimum Nuclear, Nuclear and Coal (from best to worst). If the weight is increased for a greater weight toward the impacts of workers, this ranking does not change significantly, even thought the utility of Coal decreases. If the weight of worker impacts is decreased with respect to the impacts of the public, the ranking between Coal and Nuclear change. Figure 2b illustrates the sensitivity of varying the weight given to the long-term health effects of workers in comparison to the weight given to the short- and medium-term effects. In this case, increasing the weight slightly changes the ranks of Coal and Nuclear. By contrast, Figure 2c shows how the weight given to the short-term fatal accidents for workers in comparison to short-term non-fatal accidents for workers has no impact on the final ranking of the options.

The sensitivity of the resulting ranking of the 4 options to the risk aversion factor, for the impacts of a severe nuclear accident, was also tested for the 1.2 to 2 range found in the literature review. The Figure 3 illustrates how important the choice of values for this factor can have in the final outcome of the ranking.

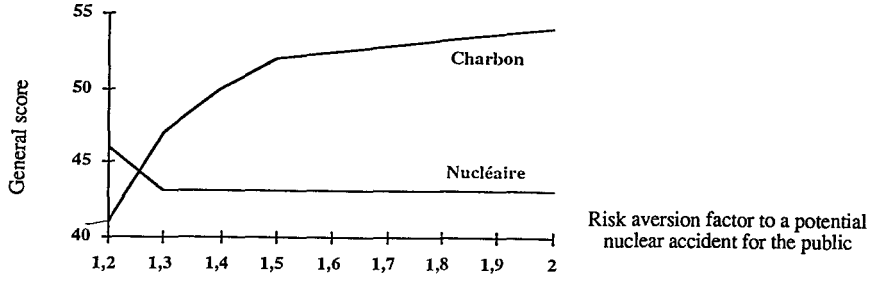


Figure 3. Impact of risk aversion factor on the score of the Coal and Nuclear Fuel Cycles.

From these preliminary results, it can be seen that the MAUA technique is useful. The sensitivity analysis has demonstrated the importance of continuing to work on determining reasonable weights for the criteria and reasonable values for the risk aversion factors. These values will be very specific to the population concerned with the ranking of the options. CEPN plans to gather information on weighting of the criteria, through further literature review and the organisation of a decision conference to determine a set of weights for a chosen group of experts. The advantage of using MAUA, it is possible to work interactively with the software and the people that are making the choices. If this is successful, it is planned that the same exercise will be used to develop weights for various other groups of people.

Institut für Energiewirtschaft und Rationelle Energieanwendung, Universität Stuttgart
Head of project : 2 Dr. habil. R. Friedrich

II. Objectives of the reporting period

IER's contribution to the project has been related to the analysis of the coal fuel cycle. Although the work effort was mainly focused on the description of human health effects, a first evaluation of environmental risk indicators was carried out to ensure a consistent integration of environmental impacts into an operational instrument supporting a decision making process. So IER's work covered four related areas of work:

1. Air pollution - human health impact pathway taking into account heavy metals.
2. Development and improvement of health risk indicators appropriate to be used for comparative risk analysis.
3. Development of a methodology allowing a consistent definition of system boundaries (with regard to up- and downstream process steps) taking into account macro-analysis techniques.
4. Compilation of environmental risk indicators used in former risk assessment studies.

Work on topics 2, 3 and 4 will be continued during the next working period. In addition, work effort will focus on the integration of health indicators describing health risks from the coal fuel cycle into the system supporting multi-attributive utility analysis currently developed at CEPN.

III. Progress achieved including publications

The results achieved during the reporting period can be summarised as follows:

1. Air pollution - human health impact pathway taking into account heavy metals.
The aim of the work in this area was to determine the importance of impact pathways related to the emissions of heavy metals. Mass balance studies reveal that coal combustion in power plants produces a selective partitioning of trace elements between various exit streams which is based on a volatilisation - condensation mechanism. Some of the trace elements remain condensed at the temperature of coal combustion and therefore immediately form a component of the inorganic ash. Other elements are volatilised and later on condense out on the

small fly ash particles. Very volatile elements like Br, Hg and I mostly remain in the gas phase during their passage through the power plant.

In a coal fired power plant, the electrostatic dust precipitator as well as the FGD-plant are considerable sinks for trace elements. Elements of health concern like lead, cadmium and arsenic were not detectable in the flue gas after FGD in mass balance studies carried out in German power plants, so that a further analysis of the relevant pathway is not possible and not necessary.

2. Development and improvement of health risk indicators

In the first phase of the project, the indicators number of fatalities, number of non fatal accidents and diseases and the number of Worker Days Lost (WDL) were used to describe occupational health risks. Based on the compilation of additional data from the German employees' industrial compensation society, the indicators "Years of Life Lost" (YOLL) and "Years of Life Impaired" (YOLI) are additionally introduced. The YOLL takes into account the life expectancy as a function of the average age at death and so allows a distinction between e.g. a fatal accident at age 35 and a fatal disease at age 70.

According to the YOLLs, the Years of Life Impaired take into account duration and degree of health damage. The degree of damage is specified according to the so called "Minderung der Erwerbsfähigkeit" (MdE) (Reduction in Earning Capacity), which is a commonly used measure of health damage in the German compensation system. YOLLs and YOLIs have been calculated for all process steps of the German coal system. Results are considered as a very helpful, complimentary information leading to a better understanding of the various health impacts.

3. Development of a methodology allowing a consistent definition of system boundaries

In doing risk assessment of energy systems, a consistent definition of system boundaries with regard to up- and downstream process steps is required to guarantee a reliable comparison of different systems. As such a definition does not exist in former risk assessment studies, a new concept combining micro and macro analysis techniques is proposed to overcome this problem.

- The *process analysis*, traditionally used in risk assessment studies, divides a complex system into well defined process steps. Risk figures are derived for each individual

process step and normalized to a unit system output. Process analysis provides process specific results with a high accuracy, but the data requirement for process analysis is enormous, so that this type of micro analysis should be applied only to the first order of the process chain.

- *Input-Output Analysis* as a tool of macro analysis techniques might be used to analyze up- and downstream process steps. Information is used that describes a whole industrial sector instead of an individual process. The specific risk normalised to a monetary unit of the final product is derived from the mass flow between industrial sectors described in monetary units related to sector specific risk figures. Preliminary calculations based on German Input-Output tables lead to the result that risks from up- and downstream process steps amount to 10 - 20 % of the risks caused by activities on the first order of the process chain.

4. Compilation of environmental risk indicators used in formes risk assessment studies

Complementary to the concept of external costs, additional indicators used for the description of environmental damage have been compiled from the literature for the purpose of integrating environmental impacts into the comparative assessment of energy systems. These indicators are:

- *Critical volume or dilution model*
  Volumes of air, soil or water which are necessary to dilute a pollutant below an allowable concentration - so called critical volumes - are calculated and compared.

- *Relative pollutant burdens*
  Ambient concentrations or depositions due to emissions of the activity under analysis are divided by relevant air quality standards, resulting in so called "relative burdens". A comparison of technologies is based on the sum of the relative pollutant burdens for the different pollutants.

- *Exceedances of critical levels and loads*
  Critical levels or loads are defined as the limits for concentrations or depositions below which no damage is expected to current knowledge. The exeedance of a critical level or load is taken as a quantitative measure of the impact to the ecosystem.

Head of Project 3: Dr. Uijt de Haag

II. Objective for the reporting period

The RIVM Institute joined the project in the beginning of 1993. For the reporting period there are two main objectives: (1) to contribute to the identification of the environmental impacts for both the coal and nuclear fuel cycles, ranging from local effects on a short time scale to global effects on a long time scale, and (2) to combine the identified environmental impacts into useful environmental indicators for use in the methodological framework for a comparative risk assessment between the coal and nuclear fuel cycles. In the definition of the environmental indicators, attention must be paid to applying a consistent approach for both fuel cycles.

In the following period the identification of the environmental impacts for the nuclear fuel cycle and coal fuel cycle will be completed and the environmental indicators will be defined.

It is also recognised that large uncertainties are associated with the assessment of both the human health impacts and the environmental impacts for the two fuel cycles. In a methodological framework for a comparative assessment of energy systems an estimation of these uncertainties is required. In the next reporting period RIVM will therefore estimate the associated uncertainties for the different steps in the impact assessment and identify the largest sources of uncertainty for further research.

III. Progress achieved including publications

To determine the environmental impacts of both the coal and nuclear fuel cycles, first we have studied the results achieved hitherto in this project, as well as in the EC/US Joint Project on the External Costs of Energy Fuel Cycles (DGXII - Joule Programme). In this way, the methodological framework for the comparative assessment was also evaluated.

The results of these projects, with respect to the identification of the environmental impacts were collected. The survey of environmental impacts is currently completed including the results of a literature search. The resulting survey of the environmental impact of both the coal fuel cycle and nuclear fuel cycle are stored in a database, identifying the emitted pollutant, the stage of the fuel cycle in which the pollutant is emitted, and the known environmental impacts. The database is set up in such a way, that it is also applicable for more general risk assessments. In the structure of the database, the impacts are organised according to the time/space matrix as applied in the methodological framework: impacts are classified to local, regional, and global impact in

the long-, medium- and short-term. The completed database will form the basis fro the definition of the environmental indicators.

It was soon recognised that in the development of useful environmental indicators. to be used in a methodological framework for comparative risk assessment, two related tasks can be distinguished. Besides a complete survey of the environmental impacts, also the demand on the environmental indicators imposed by the final use of the indicators in the framework by the decision makers have to be identified. For example, it has to be determined to what level of detail environmental indicators have to be defined, and to what extent indicators can be aggregated.

Therefore we have started to investigate the use of environmental indicators in various applications, e.g. by decision makers in the definition of the state of the environment. In this way a set of requirements is identified for a useful definition of environmental indicators in the methodological framework.

Progress Report

Contract: **FI3P-CT920064e** **Sector: C13**

Title: Studies related to the expression of the detriment associated with radiation exposure.

1) Muirhead NRPB
2) Schneider CEPN

I. Summary of Project Global Objectives and Achievements

Introduction

The primary focus of the work carried out under this contract is the development of a PC code entitled ASQRAD (assessment system for the quantification of radiation detriment). This code will provide a common framework for applying measures of radiation detriment. It is essentially complete, both containing all the commonly used health effects models and a wide range of population parameters, and having the capacity for user input of alternative data and up-dating. ASQRAD also acts as a focus for CEPN and NRPB's work on radiation detriment, since developing the system has necessitated a full review of current information, and consideration of different approaches to its quantification.

The framework of ASQRAD and its specific features have been described in previous progress reports. Here, the ongoing work carried out during this reporting period and the continuing work schedule are summarised. Furthermore, this project summary acts as a joint report for NRPB and CEPN: while the two organisations undertake separate tasks in the contract, they cooperate closely during all phases of the work.

Progress and Ongoing Work

The following topics correspond to those in the Table. It details the work schedule for ASQRAD.

General framework: This is largely complete, as the planning paper was sufficiently detailed on the whole. One area which had been causing concern was the user input options, since these are potentially complex. For instance, specifications for whole body exposure versus organ exposure have been developed, and as a result some possible user choices for a combination of health effects models would not be valid. Therefore, some additional thought was given to the desired features and their practicability. A particular concern was to keep the user choices as simple as possible. The Figure describes the approach that has now been developed. However, the user who is not fully familiar with the underlying information may still need to refer to the help and library facilities.

Review of health effects models: Two additional sets of health effects models have been added to ASQRAD: one is that produced by the US National Institutes of Health (used by ICRP in publication 60), and the other is the revised set of NRPB models to be published shortly. In addition, a review of radiation-induced breast cancer incidence from medical exposure studies has been carried out. Consideration is being given to how the data from this review may be incorporated in the system: this is not straightforward because the measures of detriment from incidence data will be different to those from mortality data.

Transferal of data across populations: As the transference of risk coefficients remains highly uncertain, it was agreed that the default assumption within ASQRAD would be that applied originally for each of the models. No additional work will be carried out on this topic.

Detriment data: A large (25 to 30) set of population parameters (population sizes, life-tables, cancer rates) has been collated. These are being refined for inclusion in the code. Work on the impairment and the quality adjustments for non-fatal effects is still being carried out.

Additional detriment measures: 'Additional' in this context means additional to the basic probability of effects and associated years of life lost from fatal effects. ICRP detailed a wide range of measures of detriment in publication 60. Some of these are more useful that others, so these are being refined to provide a range of end-points for ASQRAD. For instance, the 'conditional probability of fatal cancer' is not a particularly helpful indicator of radiation risk in itself, and therefore it will not be offered to the user.

France-UK and other comparisons: This work, to elucidate the differences in the models and assumptions used in the application of the risk projection models, is now essentially complete: its primary purpose was to clarify the procedures used in the respective CEPN and NRPB codes. However, a full description of the broader differences between the various health effects models will be necessary for the library facility.

Sensitivity analysis: This will be offered to the user when an end-point measure of detriment is reached. For each factor in the calculation, the library will suggest a range of reasonable values to aid the user. These values will be collated at the same time as the library facility is being written.

Graphics: This had been a topic of some concern since the original version of APL did not have very sophisticated graphical facilities. However, the move to a WINDOWS based system will make them more powerful (see *System coding* below). A specific set of graphics is currently being prepared.

Help/library facilities: Most of the literature has been reviewed for these, but writing of the text will not commence until the revised APL/WINDOWS version of ASQRAD has fully taken shape. A number of ways of introducing the facilities into the system have been explored. The objective is that the 'help' will be a shallow support - a few lines on that part of the system. The 'library' will provide comment on the parameters and the use of the data, give cross-references, and cite the relevant literature.

System coding: Most effort has been in this area during the reporting period. Firstly,

ASQRAD version 0.2 was rewritten in an upgraded version of APL (APL2). This also facilitated improvements to the original code. Secondly, it was decided that the system should be transferred to a WINDOWS environment. The benefit to ASQRAD is that it will be more powerful and user-friendly. This development has necessitated some alterations to the work schedule, but the integration of the system on APL/WINDOWS is now nearly complete.

User manual/ workshop preparation: The writing of the user manual will not start until 1994. In addition, it was decided that any planning for a workshop on ASQRAD should be postponed. As such, it has been included under a proposed 12 month extension of the contract - submitted to CEC in July 1993.

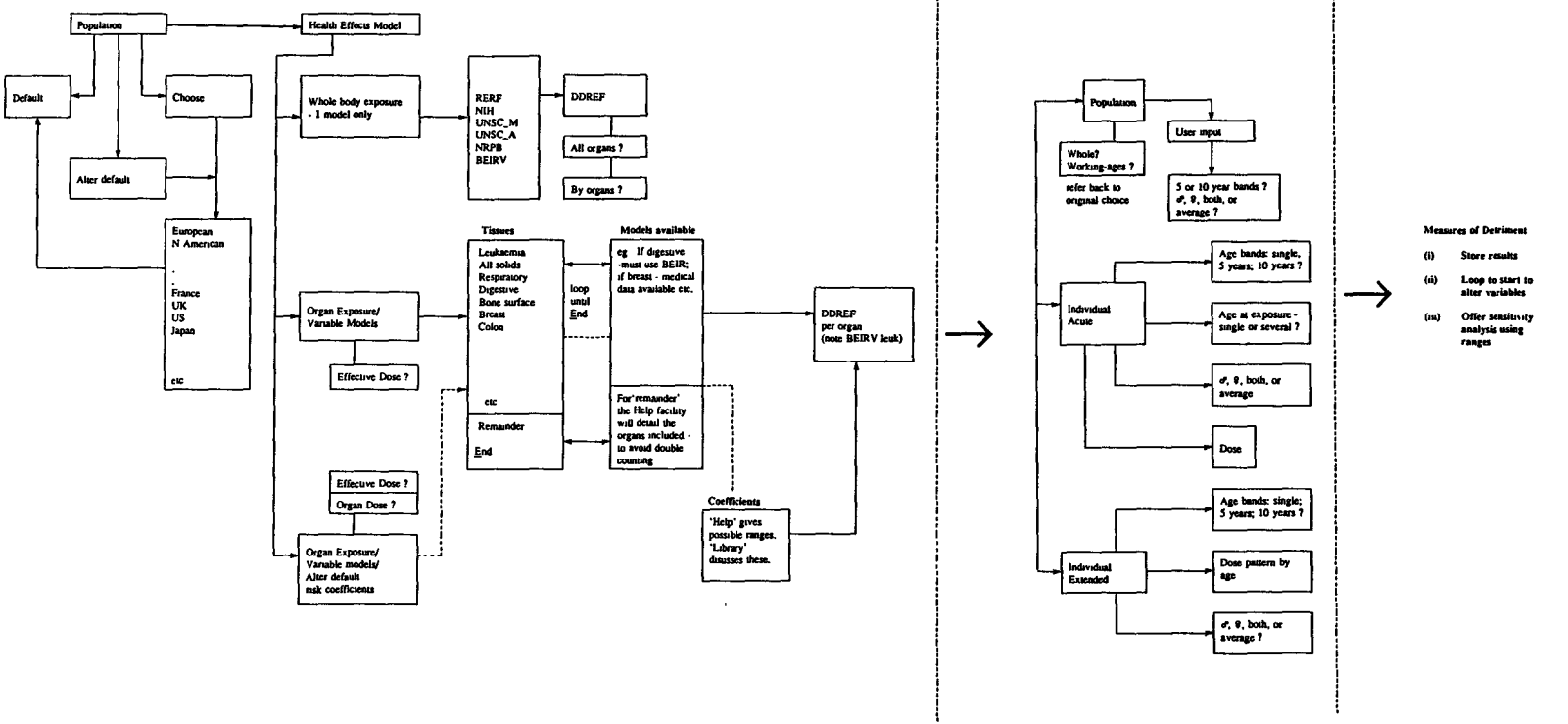
Forthcoming meeting

A contractor's meeting has been organised for 15 September 1993 at NRPB. At this, progress will be reviewed, and the work schedule for the remainder of the contract will be revised.

TABLE. **Work Schedule for ASQRAD**

TASK	TIMESCALE
1. General framework	largely complete
2. Review of health effects models - work continuing, concentrating on medical studies CEPN	Autumn 1993
3. Transferal of data across populations - defaults and additional CEPN	largely complete
4. Detriment data - impairment and quality adjustments - population parameters NRPB	ongoing 1993
5. Additional detriment measures - work continuing NRPB	1993
6. France-UK and other comparisons - focus on databases CEPN/NRPB	complete
8. Sensitivity analysis - first description completed, to be incorporated into program NRPB/CEPN	late 1993
9. Graphics - develop set of graphics and export data facility CEPN	Autumn 1993
10. Library/help facility - literature search and writing of text NRPB/CEPN	1993/94
11. System coding - first version of the main calculations already programmed - other elements still to be included - objective to produce user-friendly version with extensive graphic output CEPN	ongoing 1993/94
12. User manual/ workshop preparation NRPB/CEPN	1994

Figure Layout of user input options



Head of project 1: Dr. Kendall

II. Objectives for the reporting period

It was anticipated that NRPB's contribution to the project would be relatively minor while CEPN was recoding ASQRAD. However, work would continue on several topics. In particular, it had become clear that the user input options would be complex, and therefore a more detailed specification of this was required to ensure it would be as simple as possible. A second area would be the collation of detriment data, especially population parameters for a range of countries. Lastly, NRPB would be responsible for refining the measures of detriment made available to the user.

Objectives for the next reporting period

The joint work programme for NRPB and CEPN is summarised in the Table included with the project summary. Of these tasks, NRPB's main contribution, once the conversion of the ASQRAD code to WINDOWS is complete, will be to prepare the help and library facilities.

III. Progress achieved including publications

As expected, NRPB's contribution to this phase of the project has not been substantial while CEPN has been recoding ASQRAD. However, various important pieces of work have been carried out. One of these was the clarification of the format for user inputs. This is detailed in the Figure in the project summary. In addition, NRPB has developed a revised set of health effects models, based largely on the risk projection models prepared by the BEIR V committee. The report[1] describing these is currently awaiting publication, but the models have been implemented in ASQRAD.

Another NRPB report[2] currently awaiting publication quantifies detriment for a UK population using the NRPB models and the example measures of detriment given by ICRP in Annex C of publication 60. This report is being used as a basis for the rationalisation of the measures of detriment in ASQRAD. NRPB has also collated a wide range of age and sex specific demographic data for national populations. These data on population sizes, life-tables and cancer rates, will be refined prior to inclusion in ASQRAD.

During the reporting period, the CEC contract was transferred from the Industrial

Operations department to the Epidemiology group of the Biomedical Effects department. The project head is now Dr CR Muirhead

1. Muirhead, C R, Cox, R, Stather, J W, MacGibbon, B H, Edwards, A A and Haylock, R G E. Estimates of late radiation risks to the UK population. Chilton, Doc NRPB Vol 4 No 4 (to be published)

2. Robb, J D. 1993. Estimates of detriment in a UK population. Chilton, NRPB-R260 (to be published)

Publications

No papers have been published during the reporting period. An abstract, jointly authored by CEPN, has been submitted to the IRPA Regional Congress of June 1994.

Head of project 2: Dr. Schneider

II. Objectives for the reporting period

CEPN's contribution to the project during the reporting period was to be twofold. Their principal task was to develop the preliminary version of ASQRAD (version 0.2) prepared during the previous contract. Secondly, CEPN planned to continue their review of epidemiological studies, especially those relating to medical exposures, with a view to expanding the health effects data in ASQRAD. In particular, they would perform a specific analysis of the assumptions used to estimate breast cancer risks.

Objectives for the next reporting period

The joint work programme for CEPN and NRPB is summarised in the Table included with the project summary. Of these tasks, CEPN will continue to be responsible for the implementation of the code. Additional work reviewing health effects data will gradually tail off unless new information becomes available.

III. Progress achieved including publications

CEPN has now rewritten ASQRAD 0.2 in an upgraded version of the APL code. This has significantly improved its performance. During the reporting period CEPN also investigated the use of a WINDOWS-type PC environment to improve both the user-friendliness and the flexibility of all their software programs: the release of WINDOWS 3.1, and subsequent range of commercial software it has indirectly generated, has provided many novel opportunities for program development.

At a contractors meeting hosted by CEPN on 29 March 1993, it was agreeing that there would be advantages in also converting ASQRAD to an APL/WINDOWS environment: in particular, this will markedly simplify and enhance the user interface, the graphics, and the help and library facilities. CEPN has since been developing this approach and it is anticipated that the conversion will be complete by the end of 1993.

As far as the continued review of medical epidemiological studies is concerned, CEPN has carried out a specific analysis of radiation-induced breast cancer. This has been concerned with cancer incidence rather than mortality. A specific algorithm has been developed and it

is envisaged that this will be included in ASQRAD once appropriate measures of detriment for incidence have been discussed in more detail by the two contractors.

Publications

No papers have been published during the reporting period. An abstract, jointly authored by NRPB, has been submitted to the IRPA Regional Congress of June 1994.

Progress Report

Contract: FI3P-CT920047 **Sector:** C14

Title: Investigation of late effects in humans after artificial irradiation (Thorotrast-patients) - Follow-up study.

1) van Kaick DKFZ
2) Priest UKAEA
3) Wallin KBFOC

I. Summary of Project Global Objectives and Achievements

The intravascularly injected X-ray contrast medium Thorotrast causes life-long alpha-irradiation in the depositing organs such as liver, spleen and bone marrow. The lungs are exposed to the exhaled ^{220}Radon. From the results of the ongoing epidemiological studies in Germany and Denmark we expect contributions to high LET risk estimates for liver cancer, non-lymphocytic leukaemia, plasmocytoma, bone sarcoma and lung cancer. Furthermore we anticipate information on the late effects in the so-called non depositing organs, which are exposed to much higher doses compared to the natural external and internal radiation. These organs contain a minute amount of Thorotrast and are additionally exposed to the daughter products of ^{232}Th circulating in the blood.

We expect additional information on the observed life shortening effect by analyzing the diseases leading to death.

A recalculation of the local dose rate is urgently necessary for the assessment of the absolute risk, e.g. of the bone marrow: assessment of the distribution of Thorotrast in the yellow and the red bone marrow with regard to different bones; animal experiments to assess spatial and temporal distribution of Thorotrast in the bone marrow in comparison with autopsy results of human beings.

Mutation of the P53 gene is discussed as an early pivotal event in the etiology of many cancers. An important objective of the Thorotrast study group is to assess the frequency and the pattern of mutations in the P53 tumors suppressor gene in primary liver tumors induced by alpha-radiation from deposited ThO_2.

Head of project 1: Prof.Dr. van Kaick

II. Objectives for the reporting period

The scientific work of the clinical and epidemiological study is an extension of the program which was set up by the coordinating committee. The research activities include:

- Clinical, biochemical and radiological examinations of the Thorotrast patients and of the control group
- Biophysical examinations to calculate the tissue dose due to the thoriumdioxide (Tho_2) deposits and their radioactive daughter products
- Identification of the causes of death of Thorotrast patients and members of the control group
- Common statistical evaluation of special results of the Danish and the German epidemiological data

During the next reporting period we will continue with the work program. Additional tasks will include:

- Ophthalmological examinations to evaluate the cataract response in Thorotrast patients caused by the daughter product ^{224}Ra
- Epidemiological observation of the children of male and female Thorotrast patients and comparison with the control group
- Consideration of new dosimetric results
- Estimation of the dose to the non-depositing organs
- Investigation of specimens of autopsied Thorotrast patients by means of immunhistological and molecularbiological techniques

III. Progress achieved including publications

From May 1992 to August 1993 we examined 130 patients (57 Thorotrast and 73 control patients). The following neoplastic diseases were detected by our clinical examination (Thorotrast/control): Primary liver cancer (5/0); renal cell cancer (0/2); breast cancer (0/1); cancer of the prostate (2/1). Presently 100 Thorotrast patients are still alive.

During the reporting period 23 Thorotrast and 10 control patients have died (Table I). The extremely high proportion (70%!) of primary liver cancers causing death proves the continuing strong carcinogenic effect of Thorotrast in the liver. On the other hand, during the last years no cases of non-lymphocytic leukemias (NLL) were registered in the Thorotrast patients, which perhaps reflects the lessening leukaemogenic effect of Thorotrast.

In the control group two additional cases of cancer of the oesophagus were observed. Also another oesophageal cancer was recently diagnosed in one of our follow-up patients. By that the former excess rate of oesophageal cancer is nearly cancelled (Table II), which underlines the need to continue follow-up examinations of the control group with the same accuracy as with the Thorotrast patients.

Table I: German Thorotrast study - causes of death (05.01.92-08.01.93)

Diagnosis	Thorotrast n=23		Control n=10
Liver cancer	16		0
Liver cirrhosis	1		0
Ca oesophagus	0		2
Renal cell cancer	0	[1]	1
Lung cancer	0		1
Unknown primary	1		0
Other non-neoplastic diseases	5		6

[] Patient with liver and renal cell cancer

Table II: German Thorotrast Study - Diseases with High or Probable Excess Rate (Status 31.7.1993)

Causes of Death	Thorotrast n=2227			Control n=1527		
Liver cancer	440	[+5]*	(18.90%)	2		(0.11%)
Liver cirrhosis	190	[+176]	(15.74%)	47	[+2]	(2.59%)
Myeloid leukaemia	36	[+3]	(1.68%)	5		(0.26%)
Bone marrow failure	30		(1.29%)	5	[1]	(0.32%)
Ca ext. bil. ducts and gall-bladder	28	[+3]	(1.32%)	6		(0.32%)
Ca. pancreas	18		(0.77%)	5		(0.26%)
Ca. oesophagus	7	[+1]	(0.34%)	3		(0.16%)
Ca. larynx	6	[+1]	(0.30%)	1	[+1]	(0.11%)
Non Hodgkin lymphoma	13	[+2]	(0.64%)	3		(0.16%)
Bone sarcoma	4		(0.17%)	1		(0.05%)
Plasmacytoma	7	[+2]	(0.38%)	1		(0.05%)
Mal. Mesothelioma						
Pleural	5		(0.21%)	0		
Peritoneal	4		(0.17%)	0		

[] Additional cases with another disease leading to death
* 5 patients with combined carcinoma and sarcoma
() Inclusive additional cases related to n

Redistribution of thoriumdioxide particles in the patient's body

ThO_2 particles deposited in the organs of the RES (liver, spleen and bone marrow) can be redistributed e.g. by macrophages from spleen to liver. Until now it has been an open question as to what amount of ThO_2 is mobile and what are the consequences for the calculation of the dose rate and dose of depository organs.

During the past year an unresectable primary liver cancer was diagnosed in two Thorotrast-patients. Using imaging methods no metastatic lesions were detected. The physicians decided to perform a liver transplant. Both patients died 90 and 150 days after their respective transplants. Death was caused by diffuse metastatic lesions in the lungs and in the transplanted liver. We had the opportunity to measure the thorium-concentration in the transplanted liver. The results are summarized in Table III.

To answer the above mentioned question an animal experiment was performed two years ago. In this experiment rats were injected with Thorotrast and subsequently a Thorotrast-free liver was transplanted. Thorium-concentration was measured in the transplanted and in the original livers.

The figures of Table III show good agreement between the measurements of the Thorotrast patients and the animal data. Thorium-concentration in the transplanted liver ranged between 0.1 to 0.2 mg/g liver tissue (dry weight).

The outcome of this experiment and the biophysical measurements was that thoriumdioxide particles can be redistributed even several decades after Thorotrast injection; however, the ratio of redistributed to fixed ThO_2 is very low and will not influence significantly the calculation of the mean dose.

Table III: Original ThO_2 liver and transplanted liver of two patients and of the group G1 of an animal study.

	Time ThO_2 injection/ transplantation (y)	Injected volume (ml)	Time transplantation death (d)	^{232}Tho concentration of original liver (mg/g)	^{232}ThO concentration of donor liver (mg/g)
Pat A	44	24	90	8.5	0.16
Pat B	46	15	150	1.6	0.11
Animals G_1	0.09*	0.3**	231	1.5	0.24

* 0.09 years corresponds to approximately 2.2 years in humans

** 0.3 ml in rats coresponds to approximately 50 ml in humans

Quantification of incorporated thoriumdioxide - In vivo density and volume assessment of liver and spleen by CT

The mean calculated density values of the liver in 60 Thorotrast patients ranged from 43 to 117 HU and correlated with the incorporated Thorotrast volume (calculated by whole body counting) The correlation coefficient was r=0.69.

Using a special highlighting technique acro-agglomeration of thorium-dioxyde in the liver measuring more than 100 HU were evaluated; the relative pixel number correlated to the injected volume of Thorotrast (r=0.64).

The measurements were performed with spleen; correlations coefficient, however, have been lower than for the liver.

These results demonstrate that the mean CT density values of the liver can be used to calculate the incorporated volume of Thorotrast, especially in patients with large paravascular deposits.

In vivo volume assessment of liver and spleen was performed. The standardized liver volume was calculated based on body surface and body weight. The difference between standardized and measured liver volume was 240 g, which amounts to a decrease of about 15% of the normal liver volume in Thorotrast patients.

The mean reduction of the splenic volume was 43 g corresponding to a decrease of 30 to 40% compared to normal splenic volume.

Long time observations of more than 3 years demonstrated no significant changes of liver and spleen with regard to volume and density; only a few patients showed an increase or decrease of the density values in the liver due to fatty degeneration or organ shrinking.

Publications

Krezdorn, P. (1992) Neoplasien der Leber bei Patienten der Deutschen Thorotraststudie. Inauguraldissertation. Med. Fakultät d. Univ. Heidelberg.

Spiethoff, A., Wesch, H., Wegener, K., Klimisch, H.J. (1992) The combined and separate action of neutron radiation and Circonium dioxide on the liver of rats. Health Physics 63:111-118

Spiethoff, A., Wesch, H., Wegener, K., Klimisch, H.J. (1992) The effects of Thorotrast and quartz on the induction of lung tumors in rats. Health Physics 63:101-110

Bast, Th. (1993) Biometrische Untersuchungen bei Thorotrastpatienten mit Hilfe der Computertomographie. Inauguraldissertation, Med. Fakultät, Univ. Heidelberg

Head of project 2: Dr. Priest

II. Objectives for the reporting period

- To examine the distribution of Thorotrast within skeletal bone marrow of monkeys. The animals were injected arterially or venously and terminated after 7 d of exposure.
- To compare the red (cellular) marrow and yellow (fatty) marrow compartments.
- To compare this distribution with that found in human whole body donation Thorotrast cases.
- To examine the tissues using light microscopy, including autoradiography.
- To determine Thorium content of the samples using X-ray fluorescence (XRF) spectrometry.
- To undertake further studies on monkeys after intermediate or long term exposure.
- To produce a written report on the distribution of Thorotrast in the 7 d exposed monkeys and to make a comparison between this data and that of USUR case 1001, a human Thorotrast whole body donation.

III. Progress achieved including publications

Thorium distribution within the skeleton using XRF has been completed and comparisons made between the fatty, and red marrow distributions.

Microdistribution studies using solid state autoradiographic techniques have been undertaken and exposed autoradiographs are now being developed.

A publication is in preparation.

A set of samples has been obtained from a second Thorotrast whole body donation made to the USUR. These samples are being processed along with those obtained from Germany and Japan.

The monkeys exposed for the longer term exposed monkeys have been terminated and the tissues, both soft and osseous are being processed.

Head of project 3: Dr. Wallin

II. Objectives for the reporting period

The study is to assess the frequency and the pattern of mutations in the p53 tumor suppressor gene in primary liver tumors induced by alpha-radiation from deposited $^{232}ThO_2$ injected as Thorotrast.

Approximately 1000 Danish patients injected with Thorotrast for neuroradiological purposes during 1935-37 have been identified and followed up for incidence of cancer (1) and for pattern of mortality (2) based on data derived from the Danish cancer registry and the Danish registry of causes of death. The incidence of primary liver tumors was more than 100-fold elevated as compared with that of the general population. In a recent study based on hospital records and autopsy reports with patho-anatomic review of available histological material including up-to-date immuno-histochemical techniques liver cancer cases have been further examined and re-classified. In that study, the total number of pirmary liver cancer cases was as high as 127 (3).

Cases where suitable histological material (formalin fixed and paraffin embedded tissue blocks with malignant tissue: non-malignant tissue larger than one) available are screened for p53-mutations in exons 5-8 by constant denaturant gel electrophoresis. Mutated fragments are subsequently sequenced. The pattern of mutations will be related to estimated radiation dose, histological type of cancer, and other clinical variables.

III. Progress achieved including publications

During the reporting period the available material has been screened for tissue blocks suitable for analysis. For a total of 38 cases (19 hepatocellular carcinomas, 9 cholangiocarcinomas, 9 hemangiosarcomas, and 1 combined cholangiocarcinoma and hemangiosarcoma) qualified material was available.

From these tissue blocks sections have been sliced and de-paraffined and DNA has been extracted by standard methods. Exons 5-8 has been amplified by PCR and screened for mutations. In cases where mutations were discovered or suspected, re-screening with gel electrophoresis was performed.

Mutated fragments are currently being sequenced by standard techniques. This part of the study has been more time consuming than presumed. It is assumed, however, that it will be terminated in the near future, whereafter the results will be analyzed and reported.

The work is progressing with a minor delay in accordance to the time schedule of the technical proposal, but well within the running time of the contract.

Publications

1. Andersson M, Storm HH (1992) Cancer incidence among Danish Thorotrast-exposed patients. J Natl Cancer Inst 84:1318-1325

2. Andersson M, Juel K, Storm HH (1993) Patterns of mortality among Danish Thorotrast patients. J Clin Epidemiol 46:637-644

3. Andersson M, Vyberg M, Visfeldt J, Carstensen B, Storm HH: Primary liver tumors among Danish Thorotrast-exposed patients (in press)

Progress Report

Contract: FI3P-CT920056 Sector: C14

Title: The risk assessment of indoor radon exposure.

1)	Poffijn	Univ. Gent
2)	Tirmarche	CEA - FAR
3)	Kreienbrock	Univ. Wuppertal
4)	Kayser	Dir. Santé Luxembourg
5)	Darby	ICRF
6)	Miles	NRPB

I. Summary of Project Global Objectives and Achievements

The project consists of three major studies about the health risk of indoor radon :

- Radon and lung cancer in the Ardennes-Eifel region;
- Radon dans les habitations de Bretagne et du Massif Central et risque de cancer du poumon;
- Investigation on the relationship between lung cancer and radon in houses (UK).

The ultimate objective of the three major studies is the quantification of the risk of indoor radon exposure by means of multi-centre studies with participants in France (Centre d'Energie Atomique), Germany (University of Wuppertal), Luxemburg (Division de la Radioprotection), the United Kingdom (Imperial Cancer Research Fund and National Radiation Protection Board) and Belgium (University of Gent) .

The Ardennes-Eifel study is aimed to arrive at complete data of some 1200 cases and 3600 controls based upon a common protocol. The radon health study organised in Brittany by the C.E.A., in close collaboration with the university of Brest and INSERM, will collect complete data for some 600 cases and 1200 controls. The set-up is in complete conformity with that of the Ardennes-Eifel study. In he epidemiological study organised in Cornwall-Devon by ICRF and NRPB (with a protocol very similar to that of the two other studies of the project), a total of 600 confirmed cases of lung cancer and 1200 controls (hospital and community) , for whom a full radon history can be obtained, are aimed to be included.

The radon history of patients living at least 25 out of the last 35 years in the study area will be reconstructed by means of measurements for 6 months in the living and bedroom of all current and past houses. As different centers are using different radon devices, the comparability of the detectors and associated procedures has to be investigated through detailed quality control exercises. The radon inter-comparisons are organised by the Belgian participant (University Gent), who acts as co-ordinator for this project.

The common statistical analysis for the Ardennes-Eifel study and the Brittany study is scheduled to be performed by the German participant. The treatment of the data

will be done in close collaboration with the epidemiological research team from Oxford.

In view of carrying out a pooled analysis on a large European scale, a feasibility study will be carried out from the British Centre. In preparation of this pooling exchange of ideas with the principal investigators of ongoing and completed studies in Europe will be hold at a special meeting. At this meeting some of difficulties of achieving a common data set suitable for a pooled analysis will be discussed. Conclusions will be drawn as to the feasibility of carrying out a scientific valid pooled analysis of European studies.

After the reorganisation of the study in Luxemburg, performed in close collaboration with the other teams of the Ardennes-Eifel study, the interviewing of cases and hospital controls is now going on well in all hospital centers. The interviewing of the community control group did start, as scheduled, in Germany, Luxemburg and the U.K.

Measurements in current homes has been almost completed for all persons interviewed up to now, while a programme of measurements in the past houses was commenced in most countries.

A series of inter-comparison exercises both in laboratory and field conditions has been performed. As a consequence of this one type of detector was excluded for further use and the particular behaviour of a second device at some occasions is now being studied in detail.

In order to perform a common analysis of the data from the Ardennes-Eifel and Brittany study, a common coding schedule has been compiled. According to this scheme every centre is submitting data periodically to the co-ordinator in Gent, as a standard ascii-file. Here a data-filter is applied in order to transfer all data in an appropriate form to Wuppertal.

Meetings of the members of the group have been organised about every 6 month, in order to finalise the common approach and schedule the plans for the future. At the last meeting in Paris, it was agreed that case and control enrolment should last up to mid 1995 and that all measurements in current homes should be complete by approximately the end of 1995. Measurement in past homes will be intensified and are planned to be completed before September 1996.

In preparation of a future (potential) pooling on an European scale, all members of the group will participate in the inter-comparison exercise for epidemiology, that is scheduled to start in December 1993.

Head of project 1 : Dr. Poffijn

II. Objectives for the reporting period

The major objectives for the reporting period were :

- to interview cases and controls inthe different departments of all 5 participating hospitals;
- to perform radon measurements in the current houses of all patients;
- to set-up a general system for optimising contact with the inhabitants of past houses of the cases and controls;
- to test the data filter on a basic set of house-data delivered by the different collaborating institutes in the different participating countries;
- to draw conclusions out of the field and laboratory inter-comparison exercise organised within the Ardennes-Eifel group.

III. Progress achieved including publications

A total of 523 patients has been contacted in the 5 hospital centers. About 9% was excluded based upon the selection criteria of the common protocol and 6% refused to collaborate.

Installation of long term radon measurement devices , both in the living-room and major bedroom of the current houses of the patients recruited during the reporting period is almost completed (90%) . Installation of detectors in previous houses is in good progress, although this is much more time consuming than for the current homes. To minimise time-loss, a network of local contact persons has been set-up. An extra difficulty to deal with is the recently published restrictive law about the consultation of population registers.

The construction of the data filter is being finalised. For all members of the Ardennes-Eifel study, one or more sets of data has already been tested.

All medical data of cases recruited up to the end of 1992 have been reviewed by a medical doctor, engaged especially for this purpose.

In close collaboration with the involved teams in the different countries and with the responsibles of NRPB, quality control and performance tests for the different types of detectors used in the study by the different radon laboratories, were organised both in field and laboratory conditions. The influence of different factors and effects was studied. In general the results for the different laboratories and types of detectors are not statitistically

different. In a (very limited) number of cases some of the results of one type of detector did differ very much from the others.

The cause(s) and the influence as well as the consequencies for the common epidemiological study will be studied in detail in the coming months together with the other members of the group.

The estimation of missing radon values will be one of the principal challenges for the next reporting period. The predictive value of Po-210 as tracer for past radon exposures (project "Retrospective assessment of Radon Exposure from Long-Lived Decay Products"), will be tested on a large scale . The fact that after all only radon exposures of the past are relevant for lung cancer risk experienced today, opens perspectives for such a retrospective technique.

In preparation of a future (potential) pooling on an European scale, the radon research group of the University of Gent will participate in and co-ordinate the intercomparison exercise for epidemiology, that is scheduled to start in December 1993.

Publications :

A. Poffijn, M. Tirmarche, L. Kreienbrock, P. Kayser, S. Darby : Radon and Lung Cancer : Protocol and procedures of the multi-center studies in the Ardennes-Eifel region, Brittany and the Massif Centran Region; Radiation Protection Dosimetry, vol 45, 1-4, p. 651-656, 1992.

L. Kreienbrock, A. Poffijn, M. Tirmarche, P. Kayser, S. Darby, H.-E. Wichmann : Radon and lung cancer in the Ardennes-Eifel region - Concepts, experiences and current progress of an international epidemiological study. In : Medizinische Informatik, Biometrie und Epidemiologie 76, p. 19-23; Medizin Verlag München 1993.

A. Poffijn : Results of a pilot study on radon and lung cancer in the Belgian Ardennes; Book of abstracts Fifth International Conference of the International Society for Environmental Epidemiology, p. 32;Stockholm, Sweden, August 15-18,1993.

Head of project 2: Dr. Tirmarche

POINT OF THE CASE-CONTROL STUDY IN FRANCE AT THE END OF AUGUST 1993

AIM OF THE STUDY:

Evaluation of the risk of lung cancer linked to domestic radon exposure, measured in the houses occupied during the last 30 years.

PROTOCOL:

This study is in all points conform to the European protocol determined in this working group; it is conducted in the Ardennes region,with the main clinical center in Charleville-Mézières, being part of the international Ardennes-Eifel study. It is also extended to the region of Bretagne-Vendee, where 6 départments and 10 hospitals are involved, the main coordinating center being in Brest.

PRESENT POINT :

In the Ardennes:
At the end of august 1993, 170 interviews had been conducted and registered from the Ardennes region; out of these questionnaires, focusing on residences of the last 30 years, 450 houses have to be measured. At the present state of the study, the results from 200 dosemeters, exposed during 6 months, have been registered. Two persons are involved in the interviewing of the patients in the hospitals and two other persons are in charge of the implementation of the dosemeters in the different houses.

In the Bretagne-Vendée region:
Presently 276 interviews have been conducted corresponding to 92 triplets, isued mainly from the hospitals of Brest and Quimper; in Rennes, city at the border of the region included in this study, and because of the inclusion criteria (at least 25 years residence during the last 30 years in the precised region), the recruitment of cases and controls is low. In Nantes, coordination between the University hospital and the "anti-cancer center" was less performing than expected, but recent contacts with the main pneumologists may conclude to a better recruitment in the future. In the smaller, regional hospitals like Cholet, Vannes.... , the recruitment of lung cancers is low (10 in Cholet, 4 in Vannes).

Measurements of radon in the different houses is conducted in coordination with the University of Sciences in Brest, under the responsibility of Pr. G. Tymen. At the present point of the study, 360 results of 6 month measurements are registered.

The Kodak dosemeter used in France being an open one, the devices used in the other countries of this international collaboration being closed ones, a regular intercomparison of these different devices in constant exposure conditions and in blind laboratory development conditions are necessary in view of a common future analyses of this European study.

PUBLICATIONS:

A. Poffijn, M.Tirmarche, L. Kreienbrock, P. Kayser, S. Darby: Radon and Lung Cancer: Protocol and Procedures of the Multicenter Studies in the Ardennes-Eifel Region, Britanny and Massif Central Region. in: Radiation Protection Dosimetry, VOL 45, 1-4, 651-656,1992

M. Tirmarche
Exposition au radon et risque de cancer. Etudes épidémiologiques au niveau des mineurs d'uranium et à partir d'expositions dans les maisons.; accepté pour publication dans Radioprotection, à paraître fin 1993

Head of project 3: Dr. Kreienbrock

II. Objectives for the reporting period

The University of Wuppertal is acting as the German participant of the study.

The German team is responsible for the personnel involved in the study in his subregion(s), interviewing of cases and controls and Rn-measurements in dwellings. In addition the team from Wuppertal should analyse common datasets which are created in close collaboration with the coordinator in Gent and the team of Oxford.

III. Progress achieved including publications

In the reporting period case and control interviews were done and Rn-measurements in the participants homes were conducted. Up to now 618 interviews werde done. The same number of Rn-measurements both in living room and in bedroom of the participants home were started. Therefore the planned sample sizes are fulfilled up to now.

In the German study population 98.48 % of the male cases and 77.97 % of the male controls are ever smokers. For women these numbers are 62.07 % for cases and 45.31 % for controls.

In preparing a common data base the team of Wuppertal prepared first datasets for testing the data filter at the coordination center in Gent.

Bacause the laboratory and detector facilities used in the study are different in the participating countries a series of different intercomparisons under laboratory and under real home conditions were made. The team of Wuppertal helps in preparing a statistical analysis using analysis of variance models. In most situations laboratory and detector facilities give same results. For epidemiological purposes the facilities are comparable.

The following publications were finished or prepared in the reporting period:

POFFIJN, A., TIRMARCHE, M., KREIENBROCK, L., KAYSER, P., DARBY, S.C.: Radon and lung cancer: Protocol and procedures of the multicenter studies in the Ardennes-Eifel region, Brittany and the Massiv Central Region. Radiation Protection Dosimetry 45, 1/4 supplement, 1992, p. 651-656

KREIENBROCK, L., POFFIJN, A., TIRMARCHE, M., KAYSER, P., DARBY, S.C.: Radon and lung cancer in the Ardennes and Eifel region - Concepts, experiences and current progress of an international epidemiologic study. In: Michaelis, J., Hommel, G., Wellek, S. / Ed. Europäische Perspektiven der Medizinischen Informatik, Biometrie und Epidemiologie. Medizinische Informatik, Biometrie und Epidemiologie 76. Medizin Verlag, München, 1993, p. 19-23

KREIENBROCK, L., POFFIJN, A., TIRMARCHE, M., FEIDER, M., KELLER, G., DARBY, S.C.: Intercomparison of α-track detectors for epidemiological purposes. in preperation

Head of project 4 : Dr. Kayser

II. Objectives for the reporting period

Les objectives de base pour la période étaient les suivants :

Suite à des différents problèmes d'organisation rencontrés au Luxembourg, notamment en ce qui concerne le recrutement de patients touchés d'un cancer de poumon, une réorganisation partielle de l'étude s'est imposée. L'étude ne se limite plus strictement sur la régiondes Ardennes, mais s'étend sur tout le territoire national.

La sélection et l'interrogation du groupe des témoins de la population.

L'installation des détecteurs radon dans le living et la chambre à coucher des personnes participant à l'étude pour deux périodes de trois mois.

La participation aux exercises d'intercomparaison.

III. Progress achieved including publications

Le choix des cas qui répondent aux critères établis se fait en étroite collaboration avec le Registre Morphologique des Tumeurs du Luxembourg. Le Registre est chargé d'établir d'une part un examen cytologique exacte et d"autre part le contact entre les médecins traitants, les patients et notre enquêtrice. Puisque les patients sont rarement hospitalisées directement après les biopsies ambulatoires, toutes les enquêtes sont réalisées à la maison du patient.

Les patients du groupe des témoins hospitaliers sont actuellement recrutés dans les 2 cliniques les plus importantes de notre pays et qui sont généralement hospitalisés dans les départements de la chirurgie générale et l'orthopédie. Normalement ces patients répondent rarement aux critères d'exclusion. L'enquête auprès des témoins hospitaliers se fait chez-eux, après leur libération de l'hôpital.

Depuis début 1993, l'étude s'est également étendue sur le groupe des témoins de la population générale. Les témoins de ce groupe sont choisis de façon aléatoire à partir de l'annuaire téléphonique et non, comme prévu, des registres communaux de la population. Pour réduire le nombre de refus, les corps de sapeurs pompiers locaux sont chargés d'établir le contact entre notre enquêtrice et les personnes choisies comme témoin.

Actuellement 205 enquêtes ont été réalisées (témoins hospitaliers 70%, témoins population 12%, cas 18%). 23% des personnes interrogées appartiennent au sexe féminin. 22% des

hommes interrogés sont des non-fumeurs, tandis que chez les femmes 76% sont des non-fumeurs.

Les mesures de radon sont effectuées pendant deux périodes de trois mois dans le living et la chambre à coucher des personnes participant à l'étude épidémiologique. En moyenne les participants ont habité pendant les 35 dernières années dans 2.7 habitations. Chez 77% des personnes les mesures du radon dans leur habitation actuelle sont terminées. Une campagne pour réaliser les mesures du radon dans les habitations antérieures est en train d'être mise en oeuvre. Les moyennes arithmétiques dans les habitations mesurées actuellement s'élèvent à 188 Bq/m^3 dans les livings et à 119 Bq/m^3 dans les chambres à coucher.

Publications :

A. Poffijn, M. Tirmarche, L. Kreienbrock, P. Kayser, S. Darby : Radon and Lung Cancer : Protocol and procedures of the multi-center studies in the Ardennes-Eifel region, Brittany and the Massif Centran Region; Radiation Protection Dosimetry, vol 45, 1-4, p. 651-656, 1992.

L. Kreienbrock, A. Poffijn, M. Tirmarche, P. Kayser, S. Darby, H.-E. Wichmann : Radon and lung cancer in the Ardennes-Eifel region - Concepts, experiences and current progress of an international epidemiological study. In : Medizinische Informatik, Biometrie und Epidemiologie 76, p. 19-23; Medizin Verlag München 1993.

Head of project 5: Dr Darby

II. Objectives for the reporting period

The objectives for the current reporting period were: to interview cases of suspected lung cancer and hospital and community controls; to carry out an ongoing review of the final diagnoses of all hospital patients; to make long term measurements of the radon concentrations in as many as possible of the current houses of both cases and controls; and to commence a programme of measurement of radon in the past houses of cases and controls.

The objectives of the next reporting period are to complete the programme of interviews for cases and hospital controls, and also the diagnostic review; to continue with the programme of measurements in current and past houses; and to commence computerisation of the data and data-cleaning.

III. Progress achieved including publications

1. Study Centres

   Since the last progress report data collection at four of the five study centres (Truro, Plymouth, Torquay, and Exeter) has proceeded smoothly. Data collection at Barnstaple has been temporarily interrupted during February and March, due to illness, but is now resuming. The four year period of case enrolment at Plymouth was completed in mid-1992, and will be completed during the next three months at the other four centres. Enrolment of hospital and community controls is continuing.

2. Cases and Hospital Controls

   A total of 2833 patients with suspected lung cancer have been identified to date (see Table 1). Two hundred and eighty one (9.9%) were too ill to be approached for interview and, of the remainder, 66 (2.8%) refused to take part. One thousand one hundred and one patients had not lived in Devon or Cornwall for long enough to satisfy our residence requirements, and 1385 patients received a full interview.

   Review of hospital discharge diagnoses has been carried out for 1253 of the 1385 patients with suspected lung cancer who received a full interview. For 846 (67.5%) the final diagnosis was lung cancer. The pathological review has continued smoothly. In about 95% of those for whom the final diagnosis was lung cancer an attempt was made to obtain a microscopic diagnosis. For 68% the diagnosis of lung cancer was confirmed by histology, and for a further 12% the lung cancer was confirmed by cytology alone.

   A total of 2145 potential hospital controls have been identified (see Table 1). Sixty three patients (2.9%) were too ill to be interviewed, and of the remainder 26 (1.2%) refused to take part in the study. A total of 787 patients did not meet the residence criteria, and 1269 received full interviews.

   Review of the hospital discharge diagnoses has been carried out for 1138 of the 1269 hospital controls with full interview. For 31 patients the final diagnosis rendered them ineligible as controls, as they turned out to have diseases strongly related to smoking (cancer of bladder (7), cancer of pancreas (4), other lung diseases (2), arterial embolism or thrombosis (2), ischaemic heart disease (1), cervical cancer (2), aneurysm of iliac artery (1), duodenal ulcer (1), pulmonary tb (2), transient cerebral ischaemia (1), cerebrovascular disease (1), pneumonia (1), chronic skin ulcer due to peripheral vascular disease (1), duodenitis (1), hernia (1), tb of kidney (1), cancer of brain (1), cardiac disrythmia (1)).

3. Community Controls

For Cornwall, selection of community controls continues to be based on Family Health Services Authority (FHSA) lists, while for Devon the procedure is now based on electoral rolls.

Overall, a total of 2860 potential community controls have been selected to date, and processing is complete for 2120. A total of 179 subjects were found to be ineligible for the study (90 had died, 51 had moved away, 29 were outside the age range required for the study and 5 were found either to have been included in the study already, or else to have another family member included in the study). For 134 subjects the GP recommended that it would be inadvisable to contact the subject usually on grounds of psychiatric illness or subnormality, 12 further subjects were found by our interviewers to be too ill to take part in the study, and 98 refused to take part. Seven hundred and four subjects had not lived in Devon or Cornwall long enough to satisfy our residence requirements, and 993 had received a full interview.

The above figures include community controls selected from both FHSA lists and electoral rolls. Just considering those selected from electoral rolls, out of the 450 selected to date, 13 (2.8%) refused and 140 (31%) were in age-groups that are not of interest. Of the remainder 7 (2.4%) were found to have died, 13 (4.4%) to have moved away from the area, 76 (25.6%) were not long term residents of Devon or Cornwall, 53 (18%) have given a full interview, and the remaining 148 (49.8%) are still being processed.

4. Outstanding Interviews

The estimated numbers of full interviews outstanding are:

cases of suspected lung cancer	- 60
hospital controls	- 235
community controls	- 452

5. Measurements in Current Homes

Of the 3647 cases, hospital controls and community controls who have received a full interview, the interviewers have reported that a detector has been successfully installed in the homes of 3380 (92.7%). Eighty five subjects (22 cases, 58 hospital controls and 5 community controls) have refused to have a detector installed in their current home, 10 returned the detectors after less than two months, and detectors have not been installed in the homes of 73 patients who have moved recently, or died before a measurement could be made. A further 82 patients lived alone and either died before a measurement could be made or did not return to their original home after discharge from hospital. These addresses have been added to the list of 'Past Addresses' (see next section) and further attempts will be made to obtain measurements for them in due course. For the remaining subjects we are awaiting a report from our interviewer or data processing is not yet complete.

The interviewers have reported that 2959 pairs of detectors have been retrieved after approximately 6 months measurement, and for 2873 of these measurements have been provided by NRPB.

6. Measurements in Past Addresses

A total of 4663 addresses of previous houses in Devon and Cornwall have been passed to NRPB in eleven batches for an initial postal approach. At the present time NRPB have returned 1352 of these addresses where there has been no response from the postal approach. These will be visited by our local interviewers.

CASES OF SUSPECTED LUNG CANCER

Year - half	Full interview	Short residence	Patient refused	Patient too ill	Total
1988 - 2	43	22	9	10	84
1989 - 1	86	50	12	21	169
2	193	170	12	34	409
1990 - 1	207	188	11	40	446
2	156	137	7	50	350
1991 - 1	177	112	3	37	329
2	180	138	8	36	362
1992 - 1	172	143	4	30	349
2	114	104	0	14	232
1993 - 1[a]	57	37	0	9	103
Total	1385[b,c]	1101	66	281[d]	2833

HOSPITAL CONTROLS

Year - half	Full interview	Short residence	Patient refused	Patient too ill	Total
1988 - 2	45	19	4	1	69
1989 - 1	61	41	2	1	105
2	160	104	4	18	286
1990 - 1	204	121	7	13	345
2	167	104	4	10	285
1991 1	123	93	1	7	224
2	140	99	1	3	243
1992 - 1	143	85	1	2	231
2	127	72	1	4	204
1993 - 1[a]	99	49	1	4	153
Total	1269[b,e]	787	26	63	2145

COMMUNITY CONTROLS

Year - half	Full interview	Short residence	Subject refused	Subject too ill	GP refused	Ineligible	Total
1989-2	5	5	-	-	2	7	19
1990-1	103	94	15	2	12	47	273
2	141	105	18	2	19	25	310
1991-1	193	135	19	2	33	27	409
2	132	122	9	3	23	30	319
1992-1	172	144	16	3	25	24	384
2	168	95	20	-	19	18	320
1993-1[a]	79	4	1		1	1	86
Total	993[b]	704	98	12	134	179[f]	2120

[a, b, c, d, e, f]: Please see overleaf for notes.

Table 1. Numbers of patients with suspected lung cancer, patients with other diseases suitable for comparison purposes and community controls identified in Devon and Cornwall.

Notes for Table 1

a. Data for this period are not yet complete.

b. Totals include 22 cases of suspected lung cancer, 58 hospital controls, and 5 community controls with full interview who refused to have a detector in their current home.

c. Hospital discharge diagnoses have been reviewed for 1052 cases of suspected lung cancer with a full interview. For 715 the final diagnosis was lung cancer.

d. Includes 30 patients who died before they could be interviewed.

e. Includes 30 hospital controls with full interview, but ineligible final diagnosis.

f. Includes 90 subjects found to have died, 51 who had moved away, 29 subjects who were outside the age range required for the study, 3 subjects who had already been included in the study as cases or hospital controls, and 2 subjects who lived in households where another member had already been included in the study.

6. Publication

Gunby, J.A., Darby, S.C., Miles, J.C.H., Green, B.M.R. and Cox, D.R. Factors affecting indoor radon concentrations in the United Kingdom. *Health Phys.* 64(1):2-12; 1993.

Head of project 6: Mr Miles

II. Objectives for the reporting period

The objectives for the current reporting period were to undertake radon measurements in the current and past homes of cases, hospital controls and community controls as part of the case-control study of the effect of radon in homes in southwest England. The ideal is to measure in all the dwellings inhabited by study subjects in the last 35 years. In practice, a small percentage of the dwellings have been demolished, extensively altered or are untraceable. A realistic aim is to measure in more than 80% of the identified buildings. NRPB also provides a secretariat for the steering group for the study and administers the external funding.

The objectives for the next reporting period are to continue with the above programme of measurements and to make further attempts to obtain measurements in homes where this proved impossible earlier.

III. Progress achieved including publications

1. Measurements in current dwellings of cases and controls

   Passive radon detectors supplied by NRPB are placed in the present homes of cases and controls by the local ICRF interviewers. Pairs of detectors remain in place for six months before return to NRPB for processing. A total of some 7,500 detectors have been received back from the interviewers. The results and further data transferred from ICRF are used to calculate house average concentrations. The final result is passed back to ICRF which informs the householder of the result if requested. Details of the available results for 3,760 dwellings are given in Table 1.

2. Measurements in past dwellings of cases and controls

   The addresses of previous dwellings occupied by both cases and controls are obtained by the local ICRF interviewers and passed to NRPB. The addresses are subject to a critical review to identify those dwellings with results available from previous surveys which are removed from the following process. Letters are sent by NRPB requesting the cooperation of the present householders and the subsequent survey is undertaken by post. In cases of refusal, no reply after three letters, or other query, details are reviewed jointly with ICRF for further action. This can be a personal visit by a local interviewer, expansion or correction of address data followed by recycling through the contact programme or deferment to the end of the programme. This follow up programme is labour intensive, but necessary if measurements are to be undertaken in a high percentage of past dwellings.

   A total of 5,293 such addresses in twelve batches have been identified by ICRF and passed to NRPB. Detectors have been placed in 3,006 of these addresses (57%). Results are available for 1,919 of these homes and are detailed in Table 2.

3. Overall status of the measurement programme

The programmes to measure the radon levels in both the current and past dwellings of cases and controls continue to run smoothly and data are being accumulated at a satisfactory rate. The overall success rate in obtaining valid results in these houses, now over 70% for the first 500 or so past addresses identified, is encouraging - work continues to increase this percentage.

4. Publication

Gunby. JA, Darby, SC, Miles, JCH, Green, BMR, and Cox, DR, 1993. Factors affecting indoor radon concentrations in the United Kingdom. Health Physics 64, 2-12.

Table 1. Results from current dwellings

Parameter	Value
Number of house results available	3,760
Average radon value	62 Bq m^{-3}.
Number <100 Bq m^{-3}	3,245
Number >99 & <200 Bq m^{-3}	305
Number >199 & <400 Bq m^{-3}	142
Number >399 & <1000 Bq m^{-3}	52
Number >999 Bq m^{-3}	16

Table 2. Results from past dwellings

Parameter	Value
Number of house results available	1,919
Average radon value	55 Bq m^{-3}.
Number <100 Bq m^{-3}	1,703
Number >99 & <200 Bq m^{-3}	137
Number >199 & <400 Bq m^{-3}	47
Number >399 & <1000 Bq m^{-3}	26
Number >999 Bq m^{-3}	6

Progress Report

Contract: FI3P-CT920062 **Sector:** C14

Title: European childhood leukaemia/lymphoma incidence study.

1) Parkin IARC

I. Summary of Project Global Objectives and Achievements

The overall objective is to investigate the incidence of childhood leukaemia in Europe since 1980, and to determine whether any change in incidence which may be observable since 1985 is quantitatively associated with the estimated exposure to radiation as a consequence of the Chernobyl accident in April 1986.

The study began in 1987. It involves cancer registries in some 22 European countries (Table A1). Each collaborating centre agrees to follow a common protocol. This requires that they provide, each year, data on all recorded cases of childhood leukaemia and lymphoma occurring in the populations covered. Collation of data from the different centres and their analysis are co-ordinated by IARC. Registries send an updated file of every case registered, with details of age, date of birth, date of diagnosis, place of residence, and histological diagnosis, at annual intervals. In addition to the case-listings, the collaborators are requested to provide annual estimates of the childhood population at risk, for the same sub-national areas as those for which estimated radiation exposure information is available. The estimated excess dose of radiation due to the accident (as dose equivalents μsSv) for the first and subsequent four years post-accident has been supplied by UNSCEAR. The first report of the study, incorporating cancer data to the end of 1988, has now been published (Parkin et al., 1993).

Head of project 1: Dr. Parkin

II. Objectives for the reporting period

The objectives were to complete the data collection from participants for the period ending 31/12/90, including estimates of the childhood population at risk by single year of age. The interim analysis (to December 1989) will be updated.

For the next reporting period (1/9/93 - 31/8/94):

- A meeting of study participants is planned on 17th September 1993 to review the protocol and operational problems.

- An updated report of the results will be prepared and submitted for publication.

- Data collection will be continued, the participants should submit case and population data for the period ending 31.12.91.

- A request for adequate funding to permit participation of collaborators in Eastern Europe (including the former Soviet Union) will be submitted.

III. Progress achieved including publications

The first full report of the results of the study (follow-up to the end of 1988) were published in the European Journal of Cancer in 1993 (copy of paper attached).

Table A1 shows the status of data reception as at 1st October 1993.

This material has been used to produce an updated analysis of the results.

A draft paper, incorporating these, has been prepared (attached). This will be modified on receipt of additional analyses, and circulated to collaborators, before being submitted for publication towards the end of 1993.

TABLE A1

EUROPEAN CHILDHOOD LEUKAEMIAS/LYMPHOMAS INCIDENCE STUDY

Data received on 01/10/93

Austria	1980-91	Leukaemia/Lymphoma
Belarus, Brest Region	1980-89	Leukaemia
Gomel Region	1980-92	Leukaemia
Grodno Region	1980-89	Leukaemia
Minsk Region	1980-89	Leukaemia
Mogilev Region	1980-89	Leukaemia
Vitebsk Region	1980-89	Leukaemia
Bulgaria	-	-
Czech Republic	1980-90	Leukaemia/Lymphoma
Denmark	1980-89	Leukaemia/N.H.L.
Estonia	1980-89	Leukaemia/Lymphoma
Finland	1980-89	Leukaemia/Lymphoma
France, Bas-Rhin	1980-89	Leukaemia/Lymphoma
Dijon	1980-90	Leukaemia/Lymphoma
Doubs	1980-90	Leukaemia
Isere	1980-87	Leukaemia/Lymphoma
Lorraine	1983-90	Leukaemia/Lymphoma
PACA & Corsica	1984-90	Leukaemia/Lymphoma
ex-German Dem. Rep.	1980-90	Leukaemia/N.H.L.
ex-Germany, Fed. Rep.	1980-90	Leukaemia/Lymphoma
Hungary	1980-90	Leukaemia
Italy, Piedmont	1980-91	Leukaemia
Varese	1980-87	Leukaemia/Lymphoma
Latvia	1980-92	Leukaemia
Lithuania	1980-90	Leukaemia/Lymphoma
Netherlands	1980-90	Leukaemia
Norway	1980-90	Leukaemia/Lymphoma
Poland	1980-90	Leukaemia/Lymphoma
Russia, Moscow	-	-
St-Petersburg	1980-90	Leukaemia
Slovakia	1980-89	Leukaemia/Lymphoma
Slovenia	1980-90	Leukaemia/Lymphoma
Sweden	1980-89	Leukaemia
Switzerland, Basel	1980-88	Leukaemia/Lymphoma
Geneva	1980-90	Leukaemia/Lymphoma
Neuchatel	1980-90	Leukaemia/Lymphoma
Saint-Gallen	1980-88	Leukaemia/Lymphoma
Vaud	1980-90	Leukaemia/Lymphoma
Zurich	1980-89	Leukaemia/Lymphoma
UK, England & Wales	1980-90	Leukaemia/Lymphoma
UK, Scotland	1980-90	Leukaemia/Lymphoma

Childhood Leukaemia Following the Chernobyl Accident: The European Childhood Leukaemia–Lymphoma Incidence Study (ECLIS)

D.M. Parkin, E. Cardis and E. Masuyer (IARC), H.P. Friedl and H. Hansluwka (Austria), D. Bobev (Bulgaria), E. Ivanov (Belarus), J. Sinnaeve (CEC, Brussels), J. Augustin and I. Plesko (Czechoslovakia), H.H. Storm (Denmark), M. Rahu (Estonia), S. Karjalainen (Finland), J. L. Bernard, P. M. Carli, M.C. L'Huillier, J.M. Lutz, P. Schaffer and S. Schraub (France), J. Michaelis, M. Möhner and W. Staneczek (Germany), M. Vargha (Hungary), P. Crosignani, C. Magnani and B. Terracini (Italy), R. Kriauciunas (Lithuania), J.W. Coebergh (Netherlands), F. Langmark (Norway), W. Zatonski (Poland), V. Merabishvili (Russian Federation), V. Pompe-Kirn (Slovenia), L. Barlow (Sweden), L. Raymond (Switzerland), R. Black and C.A. Stiller (United Kingdom) and B.G. Bennett (UNSCEAR, Vienna)

The objective of the European Childhood Leukaemia–Lymphoma Incidence Study (ECLIS) is to investigate trends in incidence rates of childhood leukaemia and lymphoma in Europe, in relation to the exposure to radiation which resulted from the accident at the Chernobyl nuclear power plant in April 1986. In this first report, the incidence of leukaemia in children aged 0–14 is presented from cancer registries in 20 European countries for the period 1980–1988. Risk of leukaemia in 1987–1988 (8–32 months post-accident) relative to that before 1986, is compared with estimated average dose of radiation received by the population in 30 geographic areas. The observed changes in incidence do not relate to exposure. The period of follow-up is so far rather brief, and the study is planned to continue for at least 10 years.
Eur J Cancer, Vol. 29A, No. 1, pp. 87–95, 1993.

INTRODUCTION

ON 26 APRIL 1986, an accident occurred at the Chernobyl nuclear power plant, about 100 km west of Kiev in the Ukraine. One reactor core and part of its containment building were destroyed, allowing radioactive particles to be released into the atmosphere. Releases continued for 9 days after the accident. Exposure to populations living beyond the immediate vicinity of the plant has been extensively reviewed by the United Nations Scientific Committee on the Effects of Atomic Radiation in its 1988 report [1].

There were three successive 'plumes' of material affecting (1) the eastern part of what was then the USSR, Poland and Sweden, (2) Central Europe, especially Austria, Bavaria, northern Italy and part of Switzerland, and finally (3) Romania and Bulgaria.

Most exposure was due to radioactive iodine (^{131}I) and caesium (^{134}Cs, ^{137}Cs). Iodine, which has a half-life of about 8 days, was only important in the first weeks following the accident, while the contribution of caesium to exposure, particularly ^{137}Cs which has a half-life of 30 years, will continue to be important for many years. Humans were exposed externally, from deposition of radionuclides on the ground, and internally from the ingestion of contaminated food (e.g. milk, leafy vegetables, grains); in the first year, more than two-thirds of dose resulted from ingestion of contaminated food [1, 2].

There have been many reports on the likely long-term consequences of the accident at Chernobyl, and the appropriate methods of surveillance—reports from international organisations include those from WHO [3], the CEC [4] and IAEA [2]. These reports, and independent evaluations of likely consequences to populations outside the immediate vicinity of the accident, e.g. [5–7] suggest that any excess cancer resulting from the levels of exposure to radioactivity from the accident will be undetectable against the expected background incidence. Nevertheless, it is acknowledged that there is considerable public disquiet about the size of the risk to health. In addition, there are already reports of a raised incidence of leukaemia in young children [8], excess infant mortality [9] and excess premature births among malformed children [10] in sub-national areas where exposure to radiation from the Chernobyl accident was higher than the national average. Even if the occurrence of leukaemia cases was entirely random, their spatial and/or temporal clustering is to be expected, and so too are further reports linking such observations to the Chernobyl accident. Finally, existing knowledge of risk of cancer during the short-term period after exposure is imprecise, because the Japanese

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Revised and accepted 6 July 1992.

life-span study did not begin until 5 years after exposure to radiation from the atomic bombs. For all of these reasons, surveillance of exposed populations for the excess occurrence of malignant disease is justified, provided that it can be undertaken with due consideration of the cost and effort involved in relation to the probable statistical power of any study.

We report here the study design and preliminary results of a project to monitor incidence rates of childhood leukaemia and lymphoma in Europe, the ECLIS study.

MATERIALS AND METHODS

Radiation exposure

The estimated dose from the first year and from the first 4 years of exposure in different regions of Europe have been obtained from UNSCEAR (Table 1). In this paper, the term 'dose' is used to denote the individual committed effective dose equivalent resulting from exposure in a given time period. These estimates were based on direct measurements carried out in 34 countries in the first year. Doses from external exposures were calculated, making assumptions about average shielding by buildings and average time spent indoors. Doses from internal contaminations were based on radioactivity of various foodstuffs and assumptions about average consumption. The measurements indicated the general climatic and geographical factors which prevailed during the first year following the accident. After the first year, the contributions to external and internal doses resulting from deposited radioactive materials (mainly ^{137}Cs) were estimated from models derived by UNSCEAR from fallout measurement experience.

Average doses were estimated for all European countries. However, in several countries where the distribution of exposures was very uneven, doses were estimated for 2–4 subregions where they were more homogenous. Figure 1 shows the countries and subregions for which these dosimetric estimates were produced.

For second and subsequent years, the projected contribution to exposure from ingestion was relatively smaller than that from external radiation. The transfer factors used to calculate internal exposures were higher in the more southerly latitudes because of the more advanced stage of the agricultural cycle in spring, when the accident occurred.

Cancer data

Data on cases of leukaemia and lymphoma occurring in children aged less than 15 years are supplied from population-based cancer registries in all of the countries listed in Table 1, although for some of the geographic regions only part of the population was covered by participating registries (France, region 3; Italy, region 1; Switzerland, regions 2–4; ex-USSR, regions 3 and 4). A map and a full list of participating centres is given in Annex A. These registries record data either for all cancers, or only for paediatric cancers or leukaemia/lymphoma cases, from geographically defined populations, and use multiple source reporting, including death certificates, as recommended by IARC/IACR [11]. All participants were required to have collected data according to these criteria for a period of at least 6 years prior to the accident (i.e. since 1980), although there were two general exceptions to this condition:

(1) For some regions, important contributions to the incidence data were provided by registries which had begun subsequent to 1980, and these were included, most notably two large paediatric cancer registries in France.
(2) For certain parts of the Russian Federation, and for Belarus, special retrospective verification of recorded leukaemia cases was considered necessary.

Table 1. Radiation doses in European countries from the Chernobyl accident (estimates prepared by UNSCEAR): effective dose (μSv)

European countries	First year	0–4 years
Austria	670	1101
Bulgaria	760	900
Region 1	720	790
Region 2	800	1020
Czechoslovakia	350	440
Region 1	275	320
Region 2	355	450
Region 3	340	390
Denmark	30	55
Finland	460	730
France		
Region 3	150	210
ex-German Democratic Republic	210	340
Region 1	260	370
Region 2	340	540
Region 3	175	290
Federal Republic of Germany	130	200
Region 1	67	100
Region 2	130	200
Region 3	490	780
Hungary	230	285
Region 1	280	370
Region 2	175	203
Italy		
Region 1	374	485
Netherlands	57	90
Norway	230	330
Poland	270	370
Slovenia	620	1045
Sweden	150	270
Region 1	390	960
Region 2	85	100
Region 3	105	150
Switzerland	270	320
Region 2	315	380
Region 3	205	240
Region 4	120	145
United Kingdom	27	32
Region 1	12	14
Region 2	105	140
Region 3	190	250
ex-USSR*	260	350
Region 1	1960	2680
Region 3	445	630
Region 4	140	190

* Belarus, Estonia, Lithuania, Russian Federation.

Data are submitted each year in the form of a case listing, with, for each case: date of incidence, date of birth, sex, place and type (urban/rural) of residence, place of birth (if available), basis of diagnosis (e.g. clinical examination, haematology/cytology, histology) and diagnosis. Diagnosis was noted as site (for

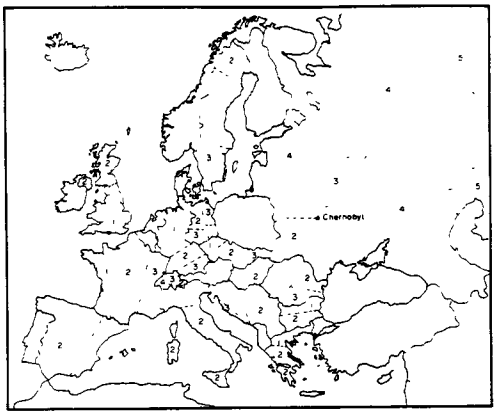

Fig. 1. Division of Europe by country, or by subregions within countries, for the purpose of dose assessment (Source: UNSCEAR, 1988 [1]). (Within a country, the numbers refer to the regions used for dose estimation, and do not imply any ranking.)

lymphomas) and morphology, and was either already coded according to the International Classification of Diseases—Oncology [12, 13], or converted from local codes to ICD-0.

The estimated size of the population-at-risk—children aged 0–14—was also supplied for each area every year, by district and type (urban/rural) of residence, sex and with maximum available detail on age (single year, if possible).

Analysis

Incidence rates were calculated for all forms of leukaemia and lymphoma, and for subgroups as defined by the ICD-0 morphology codes [14]. Rates were calculated per million person-years for age-groups 0, 1–4, 5–9 and 10–14, and the cumulative rate (0–14) was calculated as a summary index (this is effectively a direct standardisation, with the same weighting applied to each individual year of age [15]).

The main objective of this preliminary analysis was to see whether there was any evidence of a change in incidence of leukaemia in the first 3 years following the Chernobyl accident. In each country or region, the observed number of cases and incidence rate for the period 1987–1988 (8–32 months post-accident) were therefore compared with the expected value. In this preliminary analysis, the expected values were based on the average incidence rate in the 6 years pre-accident (1980–1985).

The excess risk [difference between the observed incidence rates (1987–1988) and those expected], and the excess relative risk [(observed rate/expected rate) − 1], were considered as a function of the first-year dose given in Table 1.

RESULTS

Radiation exposure

Table 1 shows the estimated radiation doses in the first year and first 4 years of exposure for the countries and subregions participating in the study. Outside the former USSR, the highest first-year dose exposures were for south-eastern, central and northern Europe. The highest country average (760 μSv) in Bulgaria is about one third of the natural background annual dose (2400 μSv), a level which corresponds with the average first-year dose in Belarus (region 1 of the former-USSR).

Leukaemia

Figure 2 illustrates the incidence of all leukaemia in children (aged 0–14) by sex in the 18 countries for which data were available for the period 1980–1985. The cumulative rates are generally in the range 400–700 per million, although somewhat lower in Poland. With a few exceptions, rates are slightly higher in boys than in girls; however, since there appears to be no difference in leukaemogenicity of radiation between the sexes, further results are presented for both sexes combined.

Acute lymphocytic leukaemia accounted for 72–83% of leukaemia cases in almost all of the registries, with the exception of Poland (54.2% in 1980–1985), where the percentage of unspecified leukaemias was also high. Since chronic lymphocytic leukaemia is extremely rare in children, and for all other forms of leukaemia the risk is known to be enhanced by exposure to radiation, further analysis was for leukaemia as a whole.

Table 2 represents some indicators of quality of data in the different countries, as a guide to interpretation of results, for the baseline period (1980–1985) and for 1987–1988. For several registries, there have been improvements in the quality of data, as reflected by these three indicators. The largest change, for Austria, is based on data from a single year (1987), for which there was a lower than expected incidence (Table 3). The data from Poland suggest some improvement in quality (lower percentages of unspecified leukaemia and DCO cases), and the possibility that some of the increase in incidence (rates were 13% higher in 1987–1988 than in 1980–1985) is due to improved ascertainment should be considered.

Table 3 presents the number of cases registered, by region, in 1980–1985 and 1987–1988, together with the cumulative incidence rates per million. The expected number of cases in 1987–1988 is also shown, based on the age-specific rates from the baseline period (1980–1985).

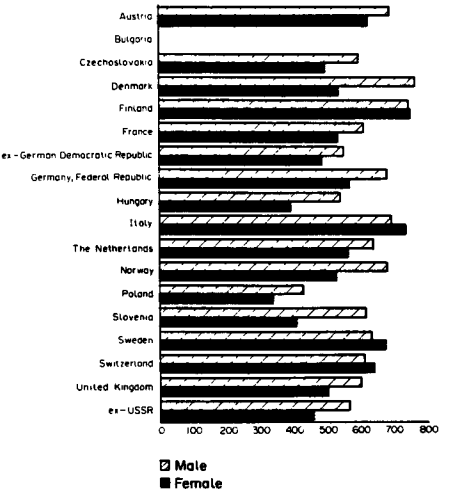

Fig. 2. Cumulative incidence of leukaemia per million, aged 0–14 years, by sex, between 1980 and 1985.

Table 2. Indicators of data quality

European countries	% Unspecified*		% Histology†		% DCO‡	
	80–85	87–88	80–85	87–88	80–85	87–88
Austria	8.3	1.9§	85.0	94.2§	14 7	5.8§
Czechoslovakia	5.9	4.8	98.7	99.0	—	—
Denmark	1.6	0.0	99.6	98.9	—	—
Finland	3.5	2.5	97.5	96.3	—	—
France	1.6	0.0	99.6	98.4	—	—
ex-GDR	0.9	3.6	98.1	99.5	—	—
FRG	0.9	1.3	84.7	95 7	—	—
Hungary	0.0	0.0	100	100	—	—
Italy	2.5	0.0	98.8	100	—	—
Netherlands	0.0	0.0	100	100	—	—
Norway	3.6	0.0§	99.0	93.1§	0.5	6.9§
Poland	19.6	11.2	69.3	67.8	5.8	3.7
Sweden	4.3	2.5	84.3	96.8	—	—
Switzerland	2.4	2.7	99.2	97.3	—	—
England and Wales	1.8	1.0	98.4	97.4	—	—
Scotland	2.3	2.1	91.6	90.6	—	—
Estonia and Lithuania	9.8	2.5	97.9	97.5	2.1	2.5
Slovenia	4.4	3.2	100	100	—	—

* Percentage of leukaemia cases of 'Unspecified' type [14].
† Percentage of cases diagnosed from bone marrow or peripheral blood.
‡ Percentage of cases registered from death certificate only (—:data not available).
§ 1987 data only.

For the entire study population for which data were available for 1987–1988, 3679 cases were observed, compared with 3533.2 expected on the basis of the 1980–1985 rates. This small increase in overall incidence (4.1%) is statistically significant ($\chi^2 = 6.0$, $P < 0.05$). However, there is no association between the change in incidence, and estimated first-year dose for the individual regions. In Fig. 3, excess risk and in Fig. 4, excess relative risk are plotted against first-year dose equivalent for the 30 countries or subregions shown in Table 3. Weighted least squares regression lines have been fitted with weights equal to 1/variance in order to diminish the effect of observations from very small populations [the two outlying points correspond to Sweden (region 2) with 9 cases in 1987/88 and Switzerland (region 3) with 4 cases]. Excess risk shows a small non-significant decline with increase in estimated unit dose ($b = -0.012, P = 0.87$), as does the excess relative risk ($b = -3.3 \times 10^{-5}, P = 0.79$).

A little over half of the leukaemia cases observed in 1987–1988 were aged less than five years at diagnosis ($n = 1923$); this is a significant increase on the number expected, based on pre-accident rates (RR = 1.066, $P < 0.01$). Once again, however, the weighted least squares regression showed no association between dose and excess risk ($b = -0.64 \times 10^{-3}, P = 0.95$) or excess relative risk ($b = +0.55 \times 10^{-5}, P = 0.98$).

DISCUSSION

Almost all studies and reports dealing with the possible consequences of the accident at the Chernobyl nuclear power plant conclude that, although a substantial number of cancers may be induced by the radioactive material released, any increase in rates outside the regions in the USSR close to the reactor site will not be detectable against the normal incidence of cancer in the general population. Using the estimate of excess relative risk of leukaemia for exposures under the age of 20 from the BEIR V report [16], and assuming that excess risk at 2–5 years is the same as that at 5–10 years (to which the BEIR data apply), we estimated that the overall increase in incidence of leukaemia for the area covered by the ECLIS study will be about 0.8% with the most marked increase in Belarus (5.8%) [17]. These estimates were, however, based on the first-year effective dose equivalent. Doses in the subsequent years would be less, with the main decline being in internal radiation. The total effective dose equivalent was estimated to be about three times that of the first-year dose [1]. For cancer cases occurring over several years, a rather more complex calculation is required, estimating the cumulated dose for each individual year of birth cohort. It is unknown, of course, whether the excess risk resulting from a dose cumulated over several years would be the same as the much higher/shorter duration exposures in Hiroshima and Nagasaki, on which the BEIR V estimates are based. However, it does seem that, unless the estimates of relative risk in relation to dose, or estimated doses, are considerably in error, no excess incidence should be detectable anywhere, with the possible exception of Belarus. It has, however, been acknowledged that surveillance is required, provided that this can be undertaken in a relatively cost-effective manner by using existing data collection systems, if only because a bland reassurance that nothing could be found is likely to be treated with scepticism by a substantial proportion of the European populations most exposed to the Chernobyl fallout.

In the context of monitoring the possible effects of exposure to low levels of radioactivity, there are several advantages to the study of leukaemia, and of cancers in childhood. Leukaemia is one of the earliest malignant neoplasms to demonstrate an increase in incidence following radiation exposure (2–10 years), and provides the largest relative increase of any cancer, at least at low to moderate exposure levels [16]. The relative risk of radiation-induced leukaemia is probably higher for those exposed as children than as adults [18, 19], and pre-natal

Table 3

	Baseline (1980–1985) Cases	Cumulative rate	Observed (1987–1988) Cases	Cumulative rate	Expected (1987–1988)* Cases
Austria	374	657	52†	588†	58 4
Bulgaria					
Region 1	Not available				
Region 2					
Czechoslovakia					
Region 1	211	526	70	555	64.5
Region 2	210	565	71	616	65.2
Region 3	405	548	149	617	132.9
Denmark	253	652	89	783	76.4
Finland	285	745	81	638	94.7
France					
Region 3	257	573	126	598	122.2
Germany					
ex-GDR					
Region 1	157	571	47	512	52.2
Region 2	143	506	51	539	47.6
Region 3	378	507	126	492	128.6
ex-FRG					
Region 1	1512	604	486	619	475.8
Region 2	471	586	161	618	151.7
Region 3	243	493	88	542	80.1
Hungary					
Region 1	222	450	88	575	66.6
Region 2	205	464	71	535	61.9
Italy					
Region 1	244	714	60	669	63.7
Netherlands	687	603	200	561	215.5
Norway	197	604	29†	550†	31.7
Poland	1431	350	516	396	498.9
Slovenia	91	515	31	558	628.2
Sweden					
Region 1	65	679	25	830	20.6
Region 2	10	500	9	1458	3.0
Region 3	320	657	123	781	103.5
Switzerland					
Region 2	67	624	20	836	15.0
Region 3	20	789	4	496	6.4
Region 4	38	562	13	578	12.7
United Kingdom					
Region 1	2076	554	701	563	688.9
Region 2	203	517	92	737	64.5
Region 3	61	636	21	679	19.7
ex-USSR					
Region 1	Not available				
Region 3					
Region 4	235	514	79	493	82.1

* Based on age-specific incidence 1980–1985.
† 1987 data only.

exposure to radiation may carry an even higher risk for childhood leukaemia [20, 21]. Some uncertainty, moreover, underlies the dose–response relationship for leukaemia in children (and adults), because of lack of data from the first 5 years following exposure in the studies of survivors of the atomic bombs in Japan. An additional consideration in the choice of childhood leukaemia was the fact that, for several countries or regions, the only cancer registries with data for the period of interest were restricted to childhood cancer (e.g. Federal Republic of Germany) or leukaemia/lymphoma (e.g. The Netherlands), or that such data were available for more extensive geographical areas, or were of better quality than comparable data for all cancers (e.g. Hungary, Austria, France). In Austria, for example, the national cancer registry files were compared sys-

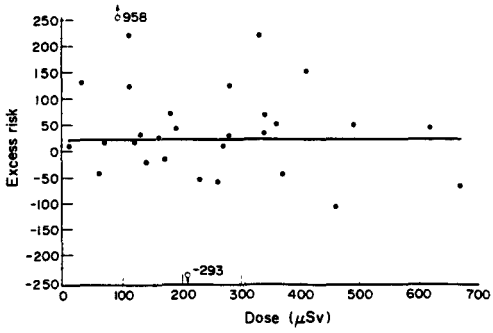

Fig. 3. Excess risk of leukaemia (observed cumulative rate − expected cumulative rate) vs. dose for 30 countries/regions (expected rate is that observed in 1980–1985).

tematically with other sources of data on childhood leukaemia (notably from clinical trials series) to produce a combined list of incident cases.

In view of the high level of contamination by ^{131}I, thyroid cancer has been a particular concern following the Chernobyl accident, and prophylactic iodide was widely administered in several countries. However, the latency period for thyroid cancer following exposure to radiation is thought to be much longer than for leukaemia. Moreover, there are problems in the uniform registration of thyroid cancer, because of the high prevalence of undiagnosed thyroid nodules in the general population, and the consequent potential for biases due to changes in the level of ascertainment. There is certainly less possibility for cases of leukaemia in the population to be unrecognised, although changes in incidence could still result from changes in the efficiency of case-finding. Although in some registries, there was evidence of improvement in some of the indicators of data quality, there was little suggestion of an association between the quality of diagnostic information and the completeness of registration, as indicated by increases in incidence data. A recent report from three districts in the Ukraine [22] suggests that enhanced surveillance and reporting of cancers after 1986 was responsible for abrupt increases in incidence of leukaemia and other cancers in the age groups 65+.

The data presented in this report relate only to childhood

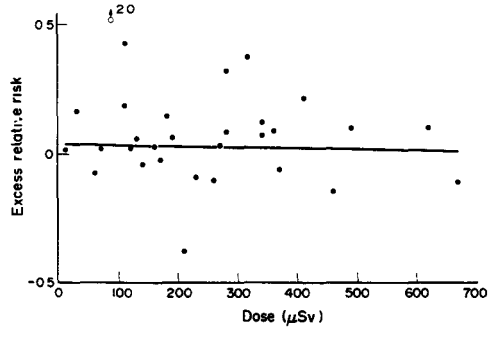

Fig. 4. Excess relative risk of leukaemia [(observed cases/expected cases) − 1] vs. dose for 30 countries/regions (expected cases calculated from rates observed in 1980–1985).

leukaemia. However, the distinction between non-Hodgkin lymphoma (NHL) and acute lymphocytic leukaemia (ALL) in childhood is somewhat arbitrary [23, 24], based upon the percentage of lymphoblasts in the bone marrow (> 25% in ALL), and NHL may progress to a leukaemic form. For this reason, data on childhood lymphoma have been collected from most centres, so that any trends in leukaemia incidence can be compared with simultaneous changes in the incidence of NHL.

The original objective of the ECLIS study was the surveillance of leukaemia (and lymphoma) incidence in countries outside the USSR. Within the former Soviet Union, a special follow-up was initiated, with a centralised register (the All Union Distributed Registry) containing medical and dosimetric information on some 530 000 individuals, including 230 000 clean-up workers, and 300 000 members of the population living in the 'special control zones' of the Russian Federation, Ukraine and Belarus which received the highest exposures [25]. It includes some 35 000 people evacuated from the zone 30 km around the reactor site, who received doses of around 400 mGy. A decision has been made recently to include incidence data from the former USSR in the ECLIS study, provided that they fulfil the criteria for the study. To date, only those from Lithuania and Estonia have been analysed. Aggregate data (numbers of leukaemia cases by age group and sex) have been received for Belarus, and do not suggest any change in incidence between 1987 and 1988 and 1980 and 1985, although since the individual records were unavailable, they are not at present included.

The data analysis comprises a comparison of population-level exposure (radiation) and outcome (leukaemia), and in common with all such ecological analyses, is subject to the limitation that cause and effect at the individual level may not be the same as those for group data [26]. However, because we are interested in changes in risk over time within areas, rather than variation between areas, the probably unequal distribution of other determinants of risk for childhood leukaemia between the different regions of Europe, for example, differences in the levels of background radiation [27], is not a concern. In this study, we are, clearly, not able to control for the change in exposure to such risk factors within the different regions over time and, as the study duration increases, the observed incidence will be based on data increasingly distant in time from the comparison, pre-accident, period. Future analyses will, therefore, examine the effect of country-specific pre-accident time trends in incidence on expected incidence post-accident.

The estimates of average radiation dose used in the analysis are those produced by UNSCEAR [1], which are in general rather similar to the earlier estimates from OECD [28]. It must be remembered that these estimates are derived from complex models involving as input measures of environmental isotopes (mainly ^{137}Cs), and a variety of assumptions about ground deposition, uptake by plants, food consumption, etc. In the absence of direct measurement, this is the only feasible methodology, although measurements close to the reactor site suggest that in the contaminated areas, the environmental transfer models (by assuming no modification of the diet) may overestimate dose [2].

In this report, we have examined data on leukaemia cases occurring up to the end of 1988 (some 32 months after the accident). This is too early to have observed any effect, even if one were anticipated at the low estimated exposure levels. At least 5 years of post-accident data are required for a meaningful analysis. At that time, it will be important to examine incidence rates specific for birth cohorts, to separate, for example, children

exposed *in utero*, and those never exposed, even *in utero*, to radiation within 12 months of the accident (born after January 1988).

The longer the timespan of the study, the more likely is it that discrepancies will arise between place of residence at time of diagnosis, and place of residence since the time of the accident. They will lead to a misclassification of populations by estimated average dose. However, with the large geographical units under consideration, there is unlikely to be much cross-boundary movement within 5 years. Its extent can be gauged by comparing the place of birth and place of residence variables in the listings of leukaemic cases, although time of migration of such cases is unknown, and it will not be possible to allocate person-years according to average level of dose.

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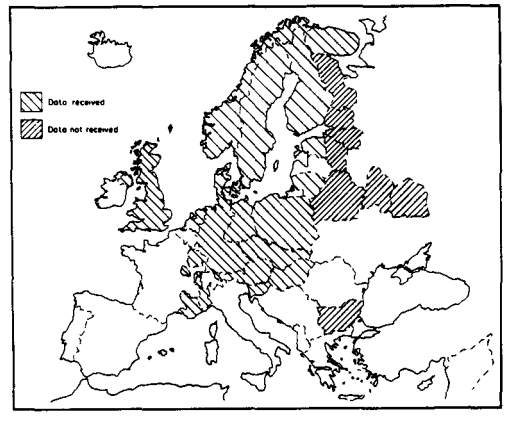

Annex A. European Childhood Leukaemia–Lymphoma Incidence Study—Coverage by geographic area.

CHILDHOOD LEUKAEMIA IN EUROPE POST-CHERNOBYL:

FOUR-AND-A-HALF-YEAR FOLLOW-UP

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INTRODUCTION

In our previous paper (Parkin et al., 1993) we described the background and rationale of the European Childhood Leukaemia-Lymphoma Incidence Study (ECLIS). Briefly, the objective is to monitor the incidence of these diseases in Europe, using existing cancer registries, to determine whether any trends observable since 1980 are associated with the excess radiation exposure which resulted from the dissemination of radioactive isotopes across Europe following the accident at the Chernobyl nuclear power plant on 26th April 1986. In this paper we describe the results of the follow-up to the end of 1990, some four years and eight months after exposure began.

MATERIALS AND METHODS

Radiation exposure

The source of data on radiation exposure consequent upon the accident was UNSCEAR, as described previously (Parkin et al., 1993). These were supplied as the estimated dose from the first year, from years 0-4, and from years 0-70 in different regions of Europe (the term 'dose' is used to mean individual committed effective dose equivalent resulting from exposure in a given time period).

The geographical units for which these estimates were produced were either whole countries or, where the distribution of exposures within a given country were uneven, for two to four sub-regions within the country. Figure 1 shows the countries and sub-regions for which the dosimetric estimates were produced.

Table 1 lists the 34 regions for which data have been contributed for the study (see below), and the corresponding dose estimates supplied by UNSCEAR.

Cancer data

Data on cases of leukaemia and lymphoma occurring in children aged less than 15 years are supplied from population-based cancer registries in all of the regions listed in Table 1, although for some of these regions only part of the population is covered, for example France (region 3), Italy (region 1), Switzerland (regions 2, 3, 4), and the former Soviet Union (region 4). At the time of the present analysis, no data have been received for Bulgaria or for the ex-USSR region 3. All together, some 35 cancer registries are participating in the study.

The Appendix contains a list of collaborating centres, and a map illustrating the areas for which data have been supplied.

The data supplied by participating registries are in the form of a case listing. The present analysis makes use of six variables: date of incidence, date of birth, sex, place of residence, basis of diagnosis, and diagnosis. In this paper, we confine analysis to cases of leukaemia. These cases were grouped into six categories, according to the international classification scheme of Birch and Marsden (1987).

Population at risk

Person-years at risk were estimated from annual mid-year estimates of the population by sex and age. Participants provided population estimates for the childhood age group, by single year of age and by sex, for their registry area or, for national registries, at a level of geographical detail sufficient to derive population-at-risk estimates for the geographical regions for which the dose estimates had been made (Table 1).

Analysis

The observed numbers of leukaemia registrations were assumed to have a Poisson distribution, with the mean proportional to the equivalent population at risk, and depending on region, age, sex, and year of diagnosis. Models with these terms were fitted using GLIM, and relative risks estimated for each of the variables.

In addition, an extra variable 'dose' was calculated for the cases and person-years at risk, and fitted in the model, to determine the effect on risk of leukaemia.

'Dose' was taken to be the leukaemogenic dose to a given child, and was estimated from the environmental 'effective dose equivalents' given in Table 1 under a series of assumptions:

1. The effect of 'environmental' exposure appears one year after exposure (latency), and lasts seven years.

2. Children aged 0 (under one year) are therefore not affected by radiation received after birth, but only by that which they received during (9 months) foetal life.

3. The effective dose to the foetus is the same as that to a free-living individual (since most of the radiation from the accident is received internally, from ^{134}Cs and ^{137}Cs ingested with contaminated food).

4. The total leukaemogenic dose is cumulative, starting with exposure at conception.

5. For convenience of calculation, the 'environmental' exposures in Table 1 were assumed to begin on 1st April 1986.

The environmental doses in the first year (1/4/86 - 31/3/87) are shown in Table 1. For the subsequent years, an exponential decay curve was fitted to the data in Table 1, so that,

Dose in years $2 \rightarrow t$ ($t = 2, 3, 4 \ldots 70$) is given by

$$\text{Dose } (2\text{-}70) \times (1 - e^{-\lambda(t-1)})$$

$$\text{where } \lambda = -1/3 \log \left(1 - \frac{\text{Dose } (2\text{-}4)}{\text{Dose } (2\text{-}70)}\right)$$

[Dose (2-4) = 4-year dose minus 1st year dose]
[Dose (2-70) = 70-year dose minus 1st year dose]

A theoretical example of the effective leukaemogenic dose received by children in relation to the estimated environmental exposures in the first five years after 1st April 1986 is shown in Figure 2.

'Dose' is therefore a function of region, year and age. It is zero for all of the cases (and population at risk) diagnosed before 1987 (total cases = 13,534). For cases diagnosed in 1987-1990 (n = 7678) the distribution of dose is shown in Figure 3.

The basic model fitted to the data, using GLIM notation, was:

AGE + YEAR + SEX + REGION

This model permits the effects of the basic variables on the risk of leukaemia to be studied. In order to evaluate the possible effect of radiation dose on risk of leukaemia, a more complex model was fitted including, in addition to the basic variables, interactions between them:

AGE . SEX + AGE . YEAR + SEX . YEAR + SEX . REGION + AGE . SEX . YEAR

These interaction terms allow, for example, for variations in time trends by sex, age (or birth cohort) and region.

By regrouping the variable 'dose' into different levels, the observed numbers of cases at different dose levels can be compared with the numbers expected, based on the above model.

RESULTS

By the end of 1990, some 21,212 cases of childhood leukaemia had been recorded, 13,534 diagnosed during the seven-year period to the end of 1986, and 7678 between 1987 and 1990. The numbers varied greatly between the different regions, from 3927 cases in UK region 1, to 23 cases in Sweden, region 2.

Figure 4 shows the relative risk by region, taking UK region 1 as the reference category. The risk of childhood leukaemia varies from a high of 1.23 in Finland, to a low of 0.68 in Poland.

Figure 5 shows the relative risk by age, taking the risk in children aged under 1 year as 1.0. The maximum risk is at ages 2-3, with a peak some 2.5 times that at age 0, and some 3.3 times that in children aged 10-14.

Risk in girls was 0.86, relative to boys.

Risk by year was studied relative to 1980 (Figure 6). The risk for the year 1990 is 1.07 (95% c.i. 1.003-1.142).

Table 2 shows observed and expected cases from the model, for two different groupings of dose. A dose of zero corresponds to cases of leukaemia diagnosed during the seven-year period 1980-1986; this baseline 'pre-accident' risk is taken as the reference category (expected cases = observed cases). Post-accident, there is no suggestion of an increase in risk by dose level. The figures broken down by age are given in Table 3.

DISCUSSION

Based upon the estimates of excess relative risk of leukaemia in children exposed to radiation from the atomic bombs in Hiroshima and Nagasaki as presented in the BEIR V report (BEIR V, 1990), we have estimated that the numbers of cases of leukaemia expected as a result of the first year exposures to radiation from Chernobyl will be too small to distinguish from the 'normal' incidence rate. The only possible exception was in Belarus, where the average first year excess effective dose equivalent (2 μSv) is about the same as the normal background radiation level, which could increase incidence by about 6%, if the BEIR V relationships hold (Parkin, 1990).

Although we observed a small increase in the risk of leukaemia over time in Europe as a whole, there did not appear to be any association between the risk of leukaemia in the period 1987-1990 and the estimated dose received. We estimated the leukaemogenic dose of leukaemia by assuming a one-year latency between exposure and effect, and that the effect of radiation would be cumulative although, as can be seen from the Figure (2) illustrating the estimation procedure, it is the first year effective dose which is largely responsible for the cumulative total in the first four years.

Although the study has been analysed as a cohort study, it should be clear that the allocation of dose to individuals is done as a function solely of place of residence. The actual exposure of individuals within the populations studied is unknown, of course, and imputing a value from the population average - itself estimated from a complex simulation model (UNSCEAR, 1988) - is subject to a large degree of misclassification.

For leukaemia cases who have moved between the regions studied between the date of the accident and the date of diagnosis, the calculated dose will clearly be incorrect; however, it is likely that interregional migrations of children in the average post-accident observation time of 2.3 years is small and will contribute only to a small extent to incorrect exposure estimation. With so few variables measured for members of the cohort, we are not able to adjust the estimated relative risks for other variables which may have been related to the exposure (itself related to region and year of diagnosis). However, it is not easy to think of possible confounding variables associated with leukaemia risk and region/year. One possibility that has been mooted - particularly in response to the claims of excess incidence rates of thyroid cancer in populations most exposed to ^{131}I contamination (Beral & Reeves, 1992; Ron et al., 1992) - is an ascertainment bias. This is the consequence of improved detection of cancer cases amongst the more heavily exposed groups. This is likely to be a problem, particularly for cancers which can exist in a 'latent' form, and be diagnosed as a consequence of an active search for them. There is no evidence that this is the case for leukaemia. A check is kept on some of the traditional indicators of data quality used by cancer registries, including the proportion of leukaemia cases of unspecified cell type and the proportion with a histological diagnosis. Apart from small improvements in the latter indicator in some of the series, there is little to suggest any

systematic increase in the completeness of registration of leukaemia cases in the most heavily exposed populations.

The findings in the present study confirm observations within the most heavily exposed population, that of Belarus (ex-USSR region 1 in Figure 1 and Table 1), where a comparison has been made of incidence rates pre- and post-accident in the heavily exposed oblasts (administrative subdivisions) of Gomel and Mogilev and the more lightly exposed (Grodno, Minsk, Vitebsk). There was no evident change in incidence in the more highly exposed populations (Ivanov et al., 1993).

In contrast, there has been a report of a strikingly high increase in the incidence of thyroid cancers, with numbers of cases observed much higher than expected, in the same areas (Kazakov et al., 1992). The validity of this observation has been questioned, partly because of the inadequate presentation of the data, and partly because of potential ascertainment bias in the post-accident observation period (Beral & Reeves, 1992; Shigematsu & Thiessen, 1992; Ron et al., 1992). The finding is also surprising because of the short latent period (previous studies of thyroid cancer induced by external radiation suggested that very much longer periods were needed), and because prophylactic iodide was administered in many populations [Belarus?] precisely to prevent the uptake of ^{131}I, which was known to be one of the radionuclides released by the accident.

The study will continue data collection for a period of seven to ten years post-accident, so that the full potential of the excess radiation exposure will be manifest. Future studies will examine in more detail the trends in incidence within different age groups and geographical regions, estimate the possible effects of migration, and study separately urban and rural populations.

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Table 1 - Radiation dose by region. Figures are effective dose (μSv) consequent upon Chernobyl accident (source: UNSCEAR)

	1 year	4 years	70 years
Austria	674	1101	2856
Bulgaria, region 1	719	791	1087
Bulgaria, region 2	796	1017	1929
Czechoslovakia, region 1	276	319	496
Czechoslovakia, region 2	355	452	853
Czechoslovakia, region 3	338	389	598
Denmark	30	54	152
Finland	463	734	1852
France, region 3	152	211	454
Germany, ex-GDR, region 1	258	370	834
Germany, ex-GDR, region 2	337	537	1357
Germany, ex-GDR, region 3	175	287	751
Germany, ex-FRG, region 1	67	104	256
Germany, ex-FRG, region 2	130	204	508
Germany, ex-FRG, region 3	488	783	1999
Hungary, region 1	279	368	733
Hungary, region 2	175	203	317
Italy, region 1	374	485	941
Netherlands	57	91	227
Norway	234	332	735
Poland	270	366	761
Slovenia	620	1045	2793
Sweden, region 1	386	959	3315
Sweden, region 2	85	100	161
Sweden, region 3	104	146	321
Switzerland, region 2	314	378	644
Switzerland, region 3	205	242	397
Switzerland, region 4	120	144	242
United Kingdom, region 1	12	14	22
United Kingdom, region 2	107	138	268
United Kingdom, region 3	191	247	475
ex-USSR, region 1 (Belarus)	1958	2675	5624
ex-USSR, region 3	445	629	1386
ex-USSR, region 4	139	190	401

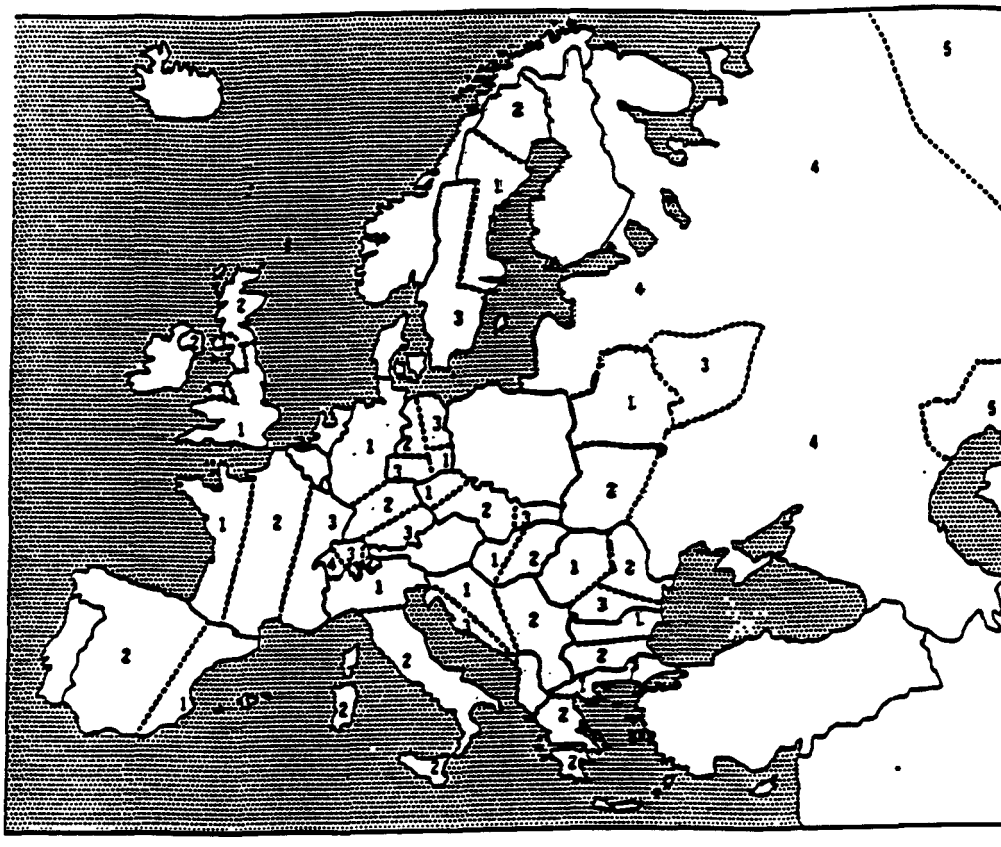
Table 2 - Numbers of cases of childhood leukaemia in Europe, by 'dose' of radiation post-Chernobyl

Cumulative dose (μSv)	Observed cases	Expected cases	Ratio
0	13 534	13 534.0	1.00
0.1- 59.9	2 790	2 779.4	1.00
60 -129.9	1 860	1 884.0	0.99
130 -299.9	1 602	1 607.0	1.00
300+	1 426	1 407.7	1.01
	20 270	20 270	

Table 3 - Observed and expected cases of leukaemia, by age group, in relation to estimated excess leukaemogenic dose

Dose	Age 0		Age 1-4		Age 5-9		Age 10-14	
	O	(E)	O	(E)	O	(E)	O	(E)
0	690	(690)	6157	(6157)	3883	(3883)	2804	(2804)
< 60	376	(391.3)	1403	(1332.3)	573	(640.6)	438	(415.1)
60-129	39	(33.0)	871	(939.2)	584	(551.2)	366	(360.6)
130-299	10	(7.1)	767	(767.0)	515	(496.2)	310	(336.7)
300+	13	(6.7)	574	(576.5)	508	(491.9)	331	(332.5)

Figure 1



Division of Europe by country, or by subregions within countries, for the purpose of dose assessment (Source:UNSCEAR, 1988). (Within a country, the numbers refer to the regions used for dose estimation, and do not imply any ranking).

Figure 2 - **ESTIMATES OF EXCESS LEUKAEMAGENIC DOSE, BY AGE AND YEAR**

Age at diagnosis	1986	1987	1988	1989	1990
14	0.0	150	430	506.3	572.5
13	0.0	150	430	506.3	572.5
12	0.0	150	430	506.3	572.5
11	0.0	150	430	506.3	572.5
10	0.0	150	430	506.3	572.5
9	0.0	150	430	506.3	572.5
8	0.0	150	430	506.3	572.5
7	0.0	150	430	506.3	572.5
6	0.0	150	430	506.3	572.5
5	0.0	150	430	506.3	572.5
4	0.0	150	430	506.3	572.5
3	0.0	150	430	506.3	510.0
2	0.0	150	430	443.9	222.4
1	0.0	150	367.6	156.2	94.1
0	0.0	87.6	79.9	27.8	24.1

YEAR OF DIAGNOSIS

Exposure (environment)	400	80	70	60	50

E.C.L.I.S. Study

DOSE

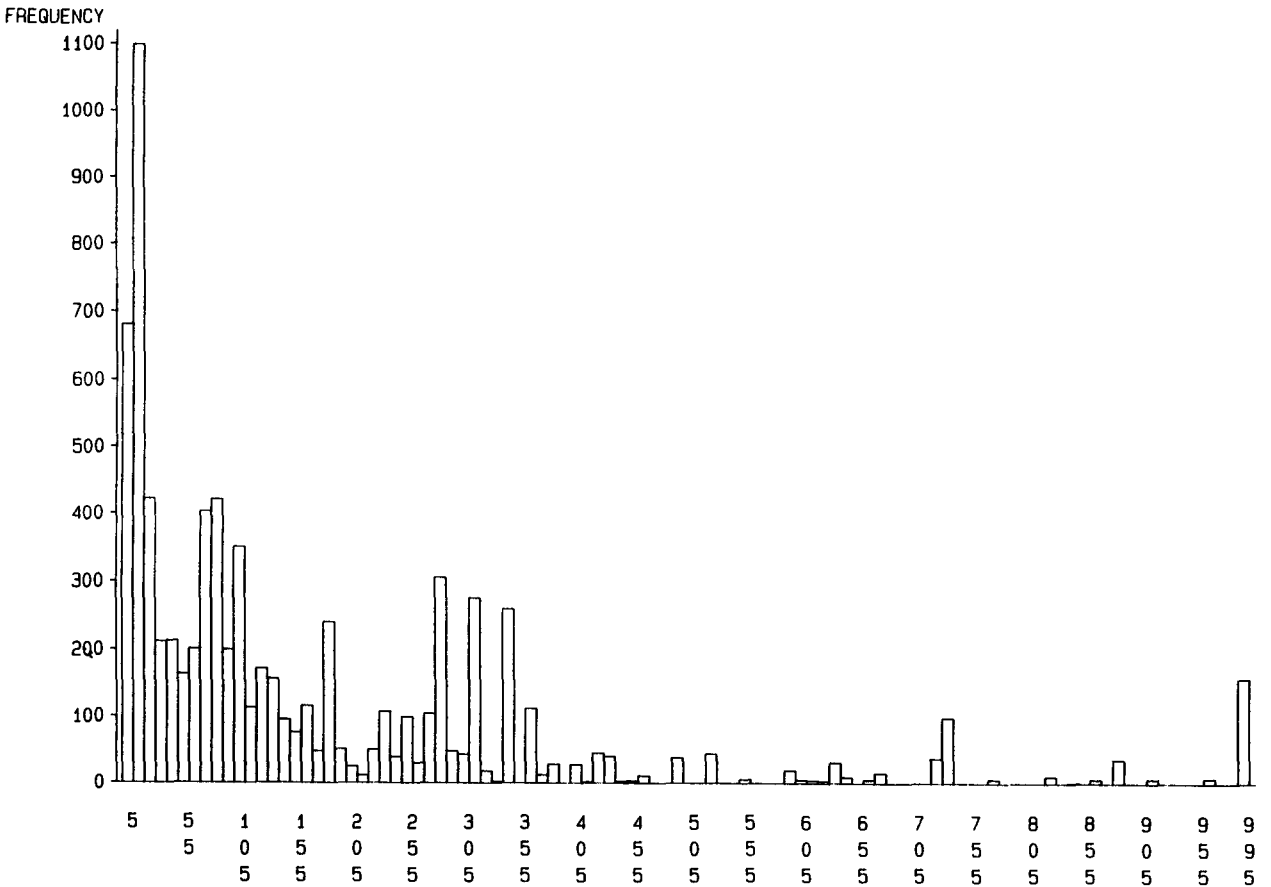


Figure 3

Figure 4

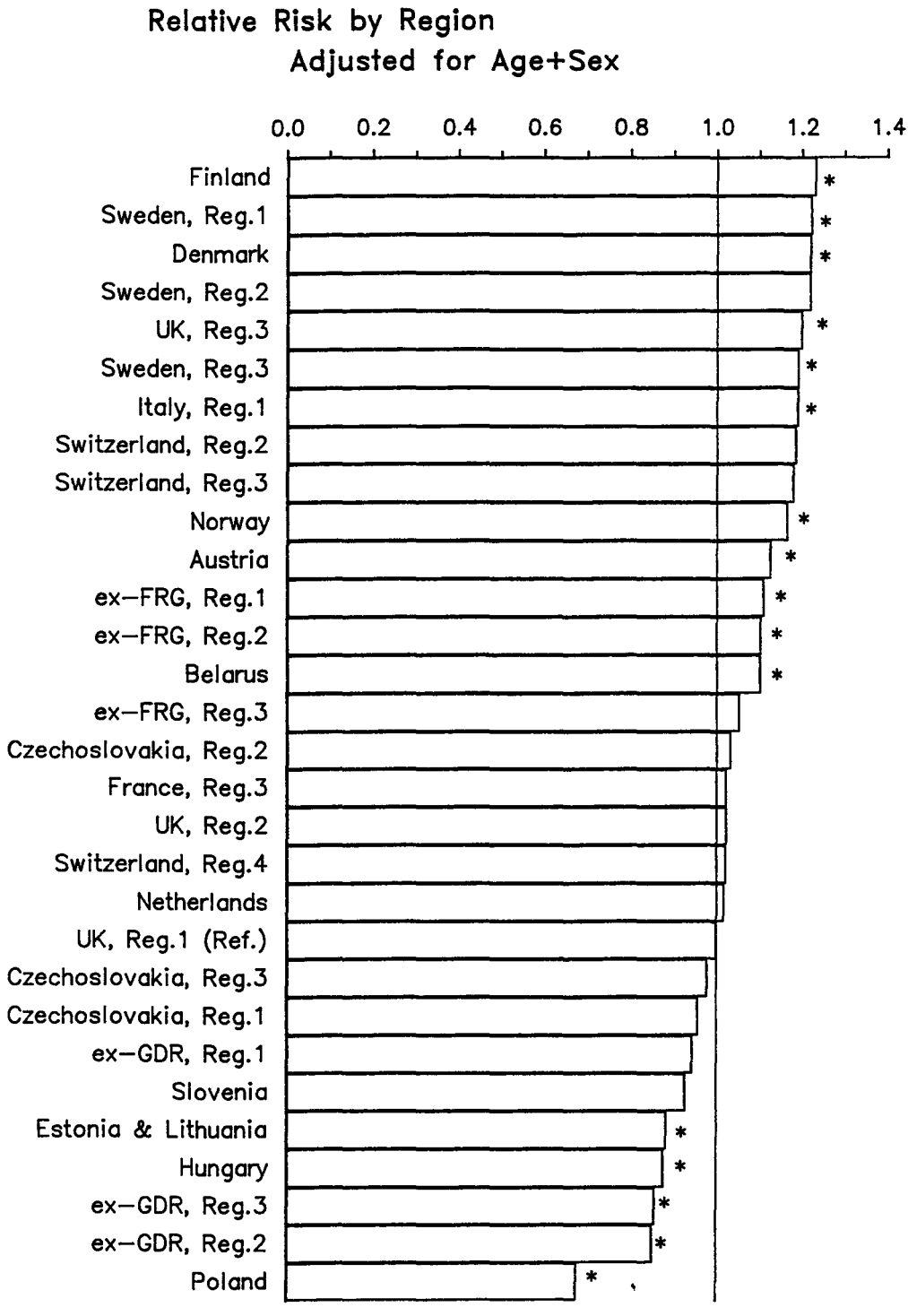

Relative Risk by Region
Adjusted for Age+Sex
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
Finland
Sweden, Reg.1
Denmark
Sweden, Reg.2
UK, Reg.3
Sweden, Reg.3
Italy, Reg.1
Switzerland, Reg.2
Switzerland, Reg.3
Norway
Austria
ex–FRG, Reg.1
ex–FRG, Reg.2
Belarus
ex–FRG, Reg.3
Czechoslovakia, Reg.2
France, Reg.3
UK, Reg.2
Switzerland, Reg.4
Netherlands
UK, Reg.1 (Ref.)
Czechoslovakia, Reg.3
Czechoslovakia, Reg.1
ex–GDR, Reg.1
Slovenia
Estonia & Lithuania
Hungary
ex–GDR, Reg.3
ex–GDR, Reg.2
Poland

Figure 5

Relative risk by age (adj. by region, sex and year)

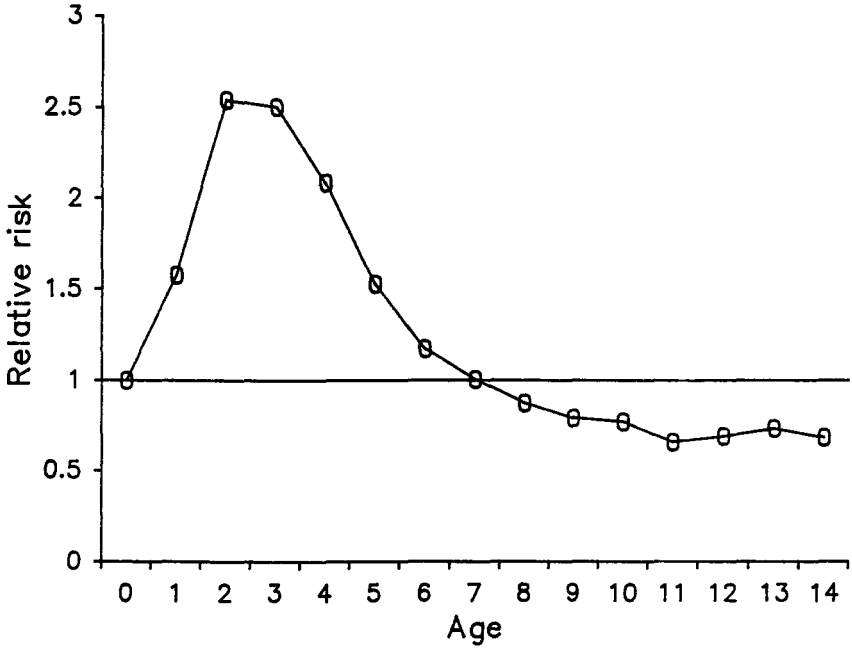
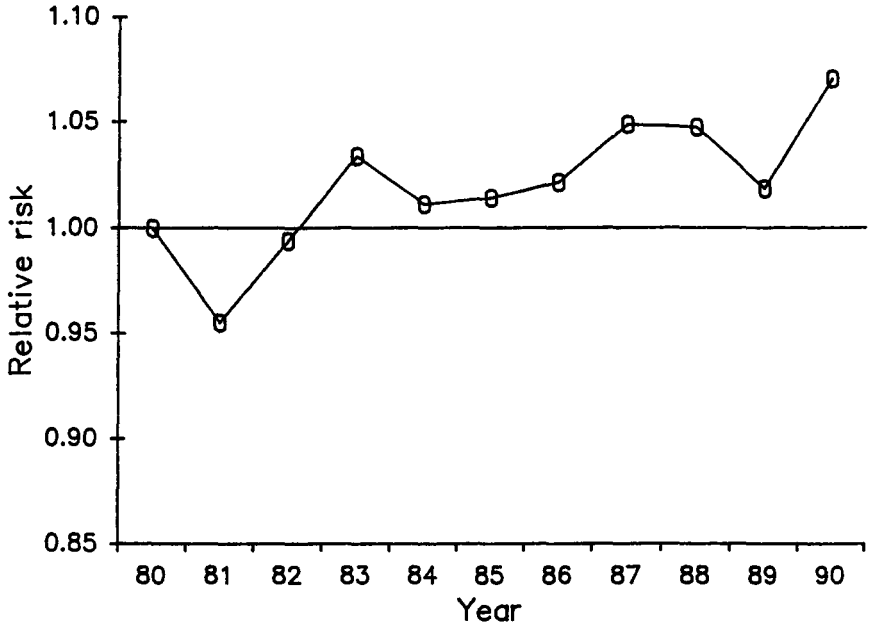


Figure 6

Relative risk by year (adj. by region, sex and age)



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Title: Epidemiological studies and tables.

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8)	Hill	Inst. Gustave Roussy
9)	de Vathaire	INSERM-U.351
10)	Wick	GSF
11)	Spiess	Univ. München
12)	Kellerer	Univ. München
13)	Kendall	NRPB

* Currently outside the contract, but expected to be included once a Memorandum of Agreement is signed between the CEC and Sweden.

I. Summary of Project Global Objectives and Achievements

The global objectives of the project are as follows:

(i) To conduct epidemiological studies and obtain information on radiation-induced cancer risks based on the following medically-irradiated cohorts:

- (a) patients with exposures to I-131 or I-123;
- (b) persons irradiated in childhood for skin haemangioma;
- (c) persons given radiotherapy for a first cancer, either in childhood or in adulthood, and followed to determine second cancer incidence;
- (d) patients injected with Ra-224.

(ii) To develop models for radiation-induced cancer risks, based on the analysis of data on populations exposed to high doses such as the Japanese atomic bomb survivors, and to examine associated methodological issues. To construct 'probability of causation' (radioepidemiology) tables that are specific to European countries. To review information on leukaemia risks and preconception irradiation.

(iii) To examine statistical methods for analysing the geographical distribution of disease. To analyse the geographical distribution of cancer in relation to natural radiation and nuclear installations.

(iv) To continue and extend a study of occupational radiation exposure and mortality, namely the UK National Registry for Radiation Workers.

Work on the project has progressed well during the reporting period. The main achievements were as follows.

Studies of Medically Irradiated Populations

At the Karolinska Institute (Sweden), detailed analyses have been performed of breast cancer risks among 9,675 women irradiated in infancy for skin haemangioma between 1920 and 1959. An excess of breast cancer was not apparent until about 40 years after first exposure; the estimated excess relative risk fifty or more years following exposure was 3.16 Gy^{-1}. There were some suggestions of a higher risk for those exposed at ages of 12-18 months than for those exposed at ages less than 6 months. Similar analyses are commencing for thyroid cancer, and discussions have been held with German researchers studying Ra-224 patients (see below) concerning methods for quantifying cataracts.

An analysis of second cancer incidence following treatment for breast cancer has been conducted at the University of Florence (Italy), based on a larger cohort than studied previously. Whereas the incidence of leukaemia in the entire cohort of 4,635 women was not raised significantly relative to regional rates, the relative risk among these women given radiotherapy was 4.7 ($p<0.05$, based on 7 cases). Follow-up of another group of patients treated for breast cancer is in progress at the University of Pisa.

Follow-up of German patients injected with Ra-224 has continued at GSF and Munich. In the early (Spiess) cohort of juveniles with bone tuberculosis and adults with ankylosing spondylitis, significant excesses of breast, liver, kidney and prostate cancer relative to standard rates have been observed. Such excesses have not been observed in a later cohort of ankylosing spondylitis patients, although in this instance there was a significant excess of leukaemia among those exposed and weaker evidence of a leukaemia excess (relative to standard rates) in an unexposed control group. A control group of non-exposed tuberculosis patients is now being constructed for the Spiess study, and visits have been made to some members of the early cohort in order to collect data on lens opacities using a Scheimpflug camera.

With regard to a study of second cancer incidence, the construction of a cohort of children treated for cancer at centres in France and Great Britain is continuing. So far about 3,600 children have been included in this study, which is being coordinated by INSERM-U.351 (France); it is hoped that the cohort will eventually number about 5,000. Preliminary analyses estimate the risk of second cancer up to 30 years following treatment to be 10% for radiotherapy alone, 21% for chemotherapy alone and 20% for both treatments. Risks were particularly high following treatment for bone sarcoma and neuroblastoma.

At the University of Saarland (Germany), a cohort of patients exposed to iodine-123 is being constructed. Since 1977, iodine-123 has been used in place of iodine-131 for thyroid examinations at this centre. It is intended to follow-up this cohort to determine their thyroid cancer risks, and to compare these risks with those in an earlier cohort exposed to iodine-131. So far, just over 10,000 patients have been included in the iodine-123 cohort.

Models and 'Probability of Causation' Tables for Radiation-Induced Cancer Risks

Models have been developed at GSF (Germany) to be used in constructing probability of causation tables for leukaemia and (in the case of radon exposures) lung cancer in European countries. These models were developed from analyses of data on the Japanese atomic bomb survivors and on Czech uranium miners respectively. Diagrams that help display these models have been produced at the University of Munich, where work has also been performed on methods for fitting time-dependent models to large datasets.

At NRPB (UK), some analyses have been published in which the Armitage-Doll model of carcinogenesis was fitted to data on groups exposed to high doses. This work is continuing with parallel analyses of data on the Japanese atomic bomb survivors and on the incidence of breast cancer

and leukaemia among women with medical exposures. The leukaemia data in particular are consistent with a three-stage model of which the first two stages are radiation-affected; this implies a linear-quadratic dose-response. Comparisons of leukaemia risks among the offspring of the A-bomb survivors and several groups of radiation workers have also been performed. As well as being incompatible with the A-bomb data, there are suggestions that the risks observed among the offspring of workers at the Sellafield plant (UK) are inconsistent with those from studies of workers in Scotland and Canada.

Geographical Studies

Researchers from Institut Gustave Roussy (France) have conducted a study in a region of northern France that is covered by a thyroid cancer registry and which contains a nuclear power plant (Chooz). No excess incidence of thyroid cancer was found in the vicinity of the plant. Further details of an analysis of mortality at ages less than 25 years around six French nuclear sites have been published in a monograph. Overall, there was no indication of excess mortality from all causes, all cancers or leukaemia around these sites. During the next reporting period, this study will be extended to cover a larger number of sites and a longer time period.

An earlier geographical correlation study of childhood leukaemia and natural radiation in Britain, performed by NRPB (UK) in collaboration with the Childhood Cancer Research Group (CCRG) (University of Oxford), has been extended to take account of socio-economic variables. No significant correlations were found between childhood leukaemia and either indoor radon or gamma dose rate using data for districts; this finding was in line with that from the earlier analysis. Further study has been made by NRPB staff of methods of detecting over-dispersion in geographically-defined disease rates; these methods have been applied to some data on childhood leukaemia incidence in parts of the USA.

Methodological work relating to the analysis of geographical correlation studies has been carried out at INSERM-U.170 (France). Modified tests of association that had been developed in an earlier CEC contract have been examined to the context of small area data. Also, the magnitude of biases that can arise in geographical studies involving two risk factors has been investigated. It is planned to apply various statistical methods to the British childhood leukaemia, natural radiation and socio-economic data mentioned above. Preparatory work for this study, which will be performed in collaboration with NRPB and CCRG, was performed during the reporting period.

Radiation Workers

Further analysis has been conducted at NRPB (UK) of data from the first follow-up of the National Registry for Radiation Workers (NRRW). In particular, the statistical significance of the trend with dose in the risk of leukaemia (excluding chronic lymphatic leukaemia) was shown to be robust to choices of lag period of 5 years or less; also, chronic myeloid leukaemia was found to be the leukaemia subtype for which the trend in risk with dose was most significant. Work is in progress to include additional groups of workers in the study, so as to increase the statistical power of the second analysis.

Meetings

The Scientific Co-ordinating Committee for this project, which comprises representatives from each of the participants, met to discuss progress on 8-9 March 1993 at NRPB Chilton (UK). The next meeting of the Committee is scheduled for 13-14 January 1994 at the University of Florence (Italy).

Heads of project 1: Dr. C.R. Muirhead
Dr. M.P. Little

II. Objectives for the reporting period

To model radiation-induced cancer risks using data on mortality among the Japanese atomic bomb survivors and on groups exposed for medical reasons. To compare the leukaemia risks among the offspring of the A-bomb survivors and among the offspring of groups of radiation workers.

To analyse the geographical distribution of childhood leukaemia in Great Britain in relation to both natural radiation and socio-economic factors. To study the properties of methods for detecting 'over-dispersion' in geographically-defined disease rates and to apply them to data on childhood cancer incidence in parts of the USA.

III. Progress achieved including publications

Modelling of Radiation-induced Cancer Risks

The analysis of the Japanese A-bomb data has continued. By way of illustrating the application of mechanistic models to describe the patterns of risk seen in radiation-exposed cohorts, a paper published by Radiation Research (Little et al, 1992) and one published in the Journal of Radiological Protection (Little, 1993a) address the fitting of Armitage-Doll models to the Japanese Life Span Study 11 data and other datasets. The second paper also summarises what is known from various high dose studies about time- and age-variation in risks and compares the population cancer risks obtained by using multistage models and time-after-exposure-adjusted relative risk models.

The application of mechanistic models to describe patterns of cancer induction by ionising radiation is continuing with parallel analyses of the Japanese mortality data in conjunction with data released by the US National Cancer Institute. The latter data concern breast cancer among women with tuberculosis who received multiple chest fluoroscopies in Massachusetts (USA) and the international case-control study of leukaemia following treatment for cervical cancer. Preliminary indications are that the breast cancer data can be modelled quite well by a model which involves a total of four stages, of which the second and third are radiation-affected. As regards leukaemia, the cervical cancer dataset has limited information on possible variation of risks by age-at-exposure; however, these data appear to be compatible with the optimal model fitted to the A-bomb survivor data. This model involves three stages, the first and second of which are radiation-affected. In particular, this implies a linear-quadratic dose-response, which agrees with what has been observed in both groups.

The full Japanese F_1 dataset, ie. that on the offspring of the Japanese atomic bomb survivors, has also been subject to some analyses. A paper examining the risks of non-cancer mortality in that dataset and comparing the risks of leukaemia in this population with those (in the study by Gardner et al, Br. Med. J., **300**, 429-434 (1990)) of the Sellafield workforce has been published in the Journal of Radiological Protection (Little, 1992). No peculiar patterns were identified in the mortality from non-malignant causes in the Japanese dataset such as might, for example, account for the discrepancies that have been observed between the leukaemia pre-conception exposure risks in that dataset and those in the Sellafield workforce. A paper in the same journal (Little, 1993b) compares the risks of leukaemia in offspring of the Ontario (McLaughlin et al. AECB INFO-0424) and the Scottish radiation workforces (Kinlen et al, Br. Med. J., **306**, 1153-8 (1993)) with those in the Japanese and Sellafield F_1 datasets. The pre-conception exposure leukaemia risks in the Japanese data are consistent with those in the Canadian and Scottish studies, but there are indications at borderline levels of statistical significance of incompatibility between the risks in the Scottish and Canadian datasets and those in the West Cumbrian data. A paper summarising and extending the previous analyses of leukaemia

pre-conception exposure risks in the four datasets, and in particular including further analysis of the effects of possible dosimetric errors in the West Cumbrian and Japanese data, has been submitted for publication in Radiation Research (Little et al., 1993).

Geographical Distribution of Childhood Cancer

An earlier analysis of childhood leukaemia and natural radiation in Great Britain did not show any statistically significant correlations based on data at the district level (Muirhead et al, Lancet, **337**, 503 (1991)). Whilst this analysis gave some indication of a positive correlation between childhood leukaemia and radon levels based on data for counties, the absence of such a relationship based on the smaller area (district) data lead the authors to conclude that the county analysis may have been affected by confounding variables. In order to check this, a joint analysis of childhood leukaemia, natural radiation and socio-economic variables has been carried out, as before in collaboration with the Childhood Cancer Research Group (CCRG) (University of Oxford). The CCRG had earlier shown in an analysis of its childhood leukaemia database (Draper et al, OPCS series SMPS No. 53 (1991)) that disease rates were higher in areas with a high socio-economic 'score' (SES). The new NRPB/CCRG analysis shows no significant correlations between childhood leukaemia and natural radiation at the county level when adjustment is made for SES. Furthermore, the district-level data continue to show no significant correlation when adjustment is made for SES. A paper describing these findings in greater detail is currently being written.

Further study is being made of a particular method for detecting whether a disease has a natural tendency to cluster. The properties of this method, derived originally by Potthoff and Whittinghill (Biometrika, 1966) and developed further by Muirhead and Butland (IARC, in press), are being examined and compared with those of some methods. This examination involves both mathematical analysis and simulations. Whilst on secondment to the Radiation Epidemiology Branch of the US National Cancer Institute (NCI), a member of the NRPB Epidemiology Group has been applying the amended Potthoff-Whittinghill method to data on childhood cancer incidence in parts of the USA covered by the NCI's SEER (Surveillance, Epidemiology and End Results) Programme. In particular, an analysis of childhood leukaemia and non-Hodgkin's lymphoma (NHL) in Connecticut showed some evidence of over-dispersion in rates for counties not containing nuclear installations, although this finding appeared to be influenced by low rates in just one county. Adjusting for this heterogeneity in the underlying disease distribution gave rise to notable increases in the p-values for the two counties in Connecticut that contain nuclear installations.

Analyses have also been performed for US data based on areas much smaller than counties, namely census tracts. However, these data were available only for some metropolitan regions, none of which contained nuclear installations. In this instance, there was very little suggestion of over-dispersion in rates for childhood leukaemia and NHL.

Publications

Little, M.P. The risks of leukaemia and non-cancer mortality in the offspring of the Japanese bomb survivors and a comparison of leukaemia risks with those in the offspring of the Sellafield workforce. J. Radiol. Prot., **12**, 203-218 (1992).

Little, M.P. Risks of radiation-induced cancer at high doses and dose rates. J. Radiol. Prot., **13**, 1-23 (1993a).

Little, M.P., 1993b. A comparison of the risks of childhood leukaemia in the offspring of the Japanese bomb survivors and those of the Sellafield workforce with those in the offspring of the Ontario and Scottish workforces, J. Radiol. Prot., **13**, 161-175 (1993).

Little, M.P., Hawkins, M.M., Charles, M.W. and Hildreth, N.G. Fitting the Armitage-Doll model to radiation-exposed cohorts and implications for population cancer risks. Radiat. Res., **132**, 207-221 (1992).

Little, M.P., Charles, M.W. and Wakeford, R., 1993. Risks of leukaemia in relation to parental pre-conception exposure to radiation. Submitted to Radiat. Res.

IV. Objectives for the next reporting period

Analyses of data on cancer risks in groups exposed to high doses will continue. In particular, it is planned to analyse data on cancer incidence up until 1987 among the Japanese A-bomb survivors, once these data have been released by the Radiation Effects Research Foundation.

A paper describing the geographical analysis of childhood leukaemia, natural radiation and socio-economic variables in Britain will be completed and submitted for publication. Papers concerning methods for detecting over-dispersion in geographically-defined disease rates and analyses of US childhood cancer data will also be prepared for publication. Further study of methodologies for analysing geographical correlations will be made in conjunction with INSERM (France) (see project 7) and, together with CCRG (Oxford), these methods will be applied to the British data described above.

Head of project 2: Prof. Dr. A.M. Kellerer

II. Objectives for the reporting period

Quantitative risk estimates of stochastic radiation effects, and especially carcinogenesis, have become increasingly important for the setting of limits in radiation protection. The estimates are obtained by the modelling of dose, age, and time dependences of excess cancer rates in large cohorts of exposed persons. One objective of the project in this reporting period has been the development of better methods of comparison of models and their application to the compilation of tables of probabilities of causation. A second major objective has been the extension of the numerical methods for maximum likelihood calculations with models that depend explicitly on the temporal distribution of exposures.

III. Progress achieved including publications

Modelling of radiation risks and construction of radioepidemiological tables

The modelling of radiation-induced cancer excess rates is usually performed with grouped data in terms of Poisson regression. The most widely used tool for the purpose is the software EPICURE with its Poisson regression subroutine AMFIT. While AMFIT is flexible and of broad applicability, it is limited by the large number of grouped data cells that are required for certain models. A particularly important case, where this difficulty is encountered, is the modelling of the dependence on the temporal distribution of radiation exposures. The relative risk models for the analysis of lung cancer rates among uranium miners are, therefore, commonly formulated either in rough steps (windows) for the dependence on time since exposure or with approximations that account for the temporal distribution by merely one or a few parameters. An alternative to the Poisson regression is the explicit person-by-person regression. In EPICURE this possibility is included through the subroutine PEANUTS. However the person-by-person regression is impracticable for large data sets, such as those for the atomic bomb survivors or, equally, the major cohorts of underground miners exposed to radon.

In view of this difficulty we have, in cooperation with the Institute for Radiation Protection of GSF, developed a hybrid algorithm for the maximum likelihood calculations. This computational procedure combines the flexibility of the person-by-person regression with the numerical facility of the Poisson regression. This permits now a substantially broader choice of models for radiation-induced excess cancer rates, including the potential for a wide class of non-parametric models that are an informative complement to the familiar models which work with pre-chosen functional dependences.

Another important aim of these studies is to achieve better criteria for the selection of models and the assessment of their equivalence or lack of equivalence. As a tool for the visualisation and quantitative assessment, and for the comparison of models we have developed plots in terms of lines of equal excess relative risk in the plane of the variable age at exposure, e, and age attained, a. The resulting e-a-diagrams have been found especially helpful in the comparison of the variety of models that have been invoked for example in the analysis of the BEIR V report but also in the largely different and often seemingly divergent approaches to the analysis of lung cancer among underground miners. In the compilation of probability of causation tables analogous e-a-plots of the probability of causation are being compiled; this has now been adopted in the project as the preferred mode of presentation of the PC-tables for their practical application.

Apart from these methodological developments considerable work in this project has gone into

the collaboration with colleagues from Branches 4 and 1 of the Institute of Biophysics in Chelyabinsk on the preparation of their reports on late health effects among the population of the Techa river and the workers of Mayak nuclear facilities. In cooperation with the Institute of Radiation Hygiene of the BfS this will be compiled and published as a special issue of *The Science of The Total Environment* (Elsevier).

Publications

Kellerer AM, Chmelevsky D, Kreisheimer M, Barclay D. A hybrid maximum likelihood algorithm for risk modelling (in preparation).

Kellerer AM, Chen J. A comparison of models for risk assessment. Asia Congress of Radiation Protection, 1993 (submitted for publication).

Kellerer AM. Risk projections in time. Proc. Symp. ICRP, Beijing, 1993 (in press).

Chmelevsky D, Barclay D, Kellerer, AM, Tomasek L, Kunz E, Placek V. Probability of causation for lung cancer after exposure to radon daughters - a comparison of models and of data. *Health Phys.*, 1993 (submitted for publication).

Head of project 3: Dr. D. Chmelevsky

II. Objectives for the reporting period

To continue work on the preparation of probability of causation (PC) tables for use in European countries, with particular emphasis on leukaemia and lung cancer.

III. Progress achieved including publications

PC tables for leukaemia

We have developed a relative risk model with a wave-like shape for the dependency on time after exposure. Based on the Hiroshima and Nagasaki mortality data it appears that one single gamma function of the above form fits the data very well. The dependency of the relative risk coefficient on age at exposure has been quantified by an exponential function. Such a relative risk model is more parsimonious than absolute risk models which have to include an additional factor for sex. They are also more convenient for the radioepidemiological (PC) tables.

PC tables for lung cancer following exposure to radon and radon daughters

We have prepared PC-tables for lung cancer following radon exposure. The background work for these tables has been a joint analysis of the S-cohort of Czech uranium miners with the Institute of Hygiene and Epidemiology in Prague. The risk model used for the PC tables represents a compromise between the risk model recommended in the BEIR IV report and results obtained from the S-cohort. In particular we have used a linear dependency on cumulated exposure. We have included smooth analytical functions (instead of the step functions used in the BEIR IV report) to account for the decrease of the risk with time after exposure and attained age.

Publications

D. Chmelevsky, D. Barclay, A.M. Kellerer, L. Tomasek, E. Kunz, V. Placek. Probability of causation for lung cancer after exposure to radon daughters - A comparison of models and of data. Accepted for publication in Health Physics.

Head of project 4: Prof. Dr. Dr. Oberhausen

II. Objectives for the reporting period

Since June 1992 we have compared our study group (patients exposed to iodine-131) with the Saarland Cancer Register in order to determine the number of patients suffering from thyroid cancer following exposure. In addition to this, we began to create our control group of patients exposed only to iodine-123 and not to iodine-131.

Most of the work in recent months was devoted to completing the control group and determining the thyroid gland-specific diagnosis of each patient at the time of exposure. We started to classify the control group-patients by their age, their sex, the number of exposures to iodine-123 for diagnostic use and their thyroid gland-specific diagnosis. We also undertook statistical analyses of data on our iodine-131 study group.

III. Progress achieved including publications

Advances made during the last six months

During the last six months we continued to assemble our control group of patients exposed to iodine-123. As well as this, we worked on the statistical analysis of the first results from our study group of patients treated with iodine-131. Since this analysis has not yet been completed, this report describes the progress made on assembling the control group up until 15.8.93. The control group has now been assembled up to the letter W as first letter of the surname.

Taking into account all criteria for inclusion (resident in the Saarland at the time of first examination, no therapy with any radionuclide, no thyroid gland-examination with iodine-131, no diagnosis of thyroid cancer at time of first examination) and exclusion, the control group up until 15.8.93 comprises 10491 patients, of whom 7566 are women and 2925 are men (corresponding to respectively 72% and 28% of the current cohort).

We divided the patients into 5-year-age groups. This classification required the exact age at time of the first examination with iodine-123 in our department. Figure 1 shows this 5-year age division, up to the last group of patients older than 85 years.

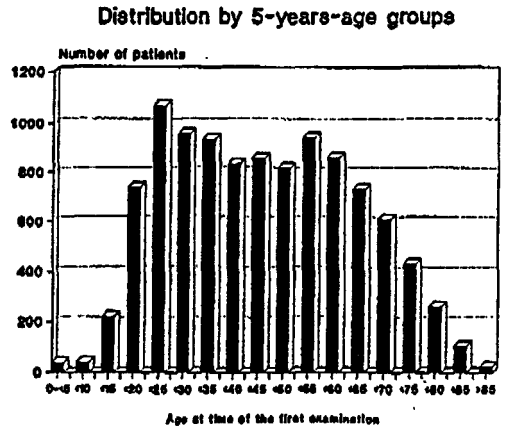

Figure 1

The period of observation begins with the change from the use of iodine-131 to iodine-123 in the second half of 1977, so until now the maximum period of observation based on full calendar years is 15 years. Patients whose first examination took place in 1992 and 1993 are characterised with 0 because they have been followed-up for less than one calendar year. Figure 2 shows the number of patients whose first examination took place fifteen (in 1977), fourteen (in 1978), thirteen (in 1979) and so on years ago.

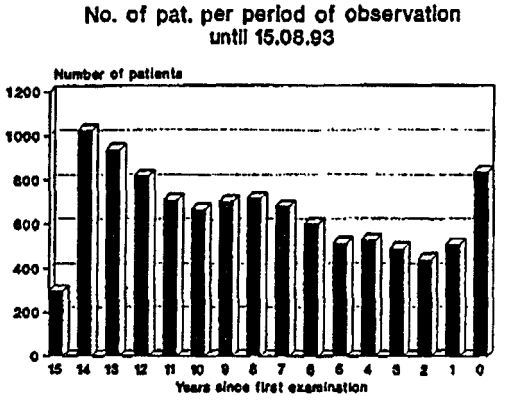

Figure 2

The majority of patients received only one thyroid gland-examination with iodine-123 in our department. If you add to these 7968 patients the 1587 patients who were examined twice, about 91% of our control group-patients received at most two thyroid gland-examinations with iodine-123. Only 1% were examined more than five times (see Figure 3). The injected activity per examination was between 7.4 and 10 MBq.

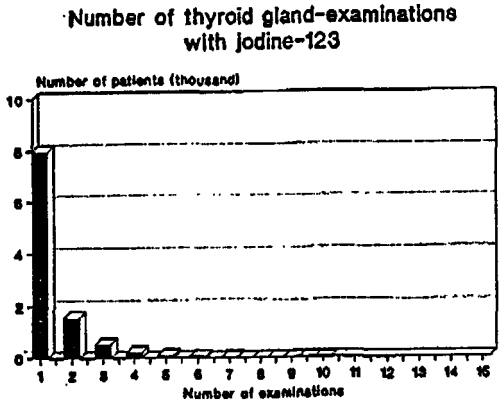

Figure 3

Because organ dose depended on the thyroid gland-diagnosis and its size, we are working on separating the patients by their diagnosis to take this into consideration.

We will soon be able to follow-up our control group using the residents' registration offices of the Saarland. We will also compare our group with the Saarland Cancer Register. Finally, we will compare our results with those for our study group.

IV. Objectives for the next reporting period

Our first objective is to complete the collection of patients into the control group. In addition, we have to determine the thyroid gland-specific diagnosis of each patient, in order to separate out those patients who were suffering from thyroid cancer at the time of the first examination.

Meanwhile we will start to check on patients' addresses using the registration offices of the Saarland. After finishing this task, we will be able to compare our control group of iodine-123-examined patients living in the Saarland with the Saarland Cancer Register, in which every Saarland resident suffering from any kind of cancer is registered. Having determined the number of patients suffering from thyroid cancer, we will compare this control group with our study group of patients exposed to iodine-131.

All these comparisons will consider the age, the sex, the amount of injected iodine and the thyroid gland-specific diagnosis of each patient.

Proposal Title and No: Cancer Incidence and Mortality Following Radiotherapy for Skin Hemangioma in Childhood.

Name of Participating Organisation: Karolinska Institute, Department of Cancer Prevention, S-104 01 Stockholm, Sweden

Head of Project: Lars-Erik Holm, M.D, Ph.D
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Aims of Project: To study cancer incidence and mortality in relation to absorbed radiation doses.

Specific Objectives for Reporting Period: To relate cancer incidence to radiation absorbed doses and to study dose-response relationships.

Progress Achieved During Reporting Period: We have been particularly interested in cancer incidence after exposure to low doses and have studied which projection model has the best fit. Ms Marie Lundell is analysing the data as part of her doctoral thesis in radiophysics. An emphasis has been made on analyses of breast cancer risk and to some extent thyroid cancer risk in relation to radiation doses.

Breast cancer
Among the 9,675 women treated as infants between 1920 and 1959, more than 50% were treated during the 1940s. On average, they were treated 1.6 times (range 1-37). There was a small variation in absorbed breast dose during the decades. The mean

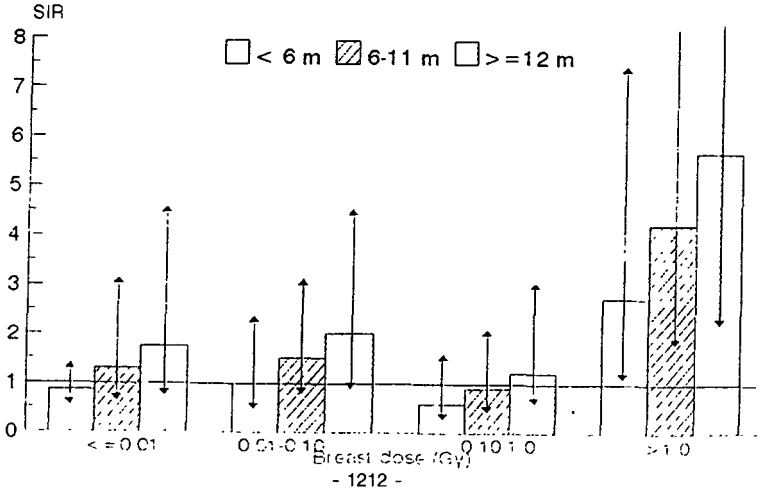
absorbed dose to both breasts was 0.39 Gy (range <0.01-35.8 Gy), and the doses to the two breasts did not differ. The mean dose was 20% higher for children <6 months old at the time of treatment than for those aged 12-18 months.

The effects of age at exposure, absorbed dose, latency period, and treatment period as factors influencing the cancer risk have been analyzed with the statistical package GLIM (Generalized Linear Interactive Modelling). The differences in deviance between models have been compared with corresponding differences in the degrees of freedom using chi-square distribution. Absorbed doses have been divided into four groups (<0.01, 0.02-0.1, 0.11-1.0, and >1.0 Gy), treatment age into three classes (<6, 6-11, and 12-18 months), treatment period into four classes (1920-29, 1930-39, 1940-49, and 1950-59), and the observation time has been divided into four classes (<30, 30-39, 40-49, and >50 years).

The cohort has been matched with the Swedish Cancer Registry for the period 1958-1986. During this period, a total of 75 breast cancers were reported in 74 women, and standardized incidence ratio (SIR) was 1.24 (95% CI: 0.99-1.55). The mean age at diagnosis was 44 years (range 25-63 years) and there were 546,705 breast years (BY) at risk.

An excess of breast cancers was not apparent until about 40 years after first exposure. For an observation time of 50 years

Dose specific, age at exposure adjusted RR for breast cancer



or more, SIR was 2.04 (95% CI: 1.17-3.32), the excess relative risk (ERR) was 3.16 per Gy, and the excess absolute risk was 30.2 per 10 BYGy .

When breast cancer risk was related to absorbed dose, the crude risk increased for all levels except for 0.11-1.0 Gy. This trend remained after adjustment for age at exposure (Figure).

The oldest group of infants (12-18 months old) had a RR of 1.90 (95% CI 1.15-3.16) versus RR=0.93 (95% CI: 0.65-1.34) for those <6 month old. This difference remained after adjustment for dose.

The ovaries of many infants were also exposed to ionizing radiation, and the mean ovarian dose was 0.05 Gy (range <0.01-8.55 Gy). The youngest group had a mean ovarian dose of 0.07 Gy (range <0.01-8.55 Gy) compared to 0.03 Gy (range <0.01-0.68) for the oldest group. Further analyses of the impact of ovarian irradiation on breast cancer risk will be carried out.

Thyroid cancer

In the total cohort of both men and women (N=14,351), 17 thyroid cancers were reported to the Swedish Cancer Register during the period 1958-1986. For the total cohort, SIR was 2.28 (95% CI: 1.33-3.65), EAR was 0.90 per 10 PYGy , and ERR was 4.92 per Gy. A considerable period at risk was truncated before 1958 for those treated in the 1920s and the 1930s, and therefore an unknown number of thyroid cancer probably occurred prior to 1958 when the cancer register was started.

We intend to analyze thyroid cancer risks more in detail, and will continue with analyses of risk for leukemia.

Cataract

We have established collaboration with the Department of Ophtalmology at Karolinska Hospital with the purpose of studying cataract in subjects irradiated for hemangioma in childhood. We intend to start inviting subjects for a clincal examination of the eyes at the beginning of the autumn of 1993. We will restrict the examination to subjects residing in the Stockholm county, and intend to use Lens Opacities Classification Systems II to study cataract.

Discussions have also begun with Dr. Kellerer in Munich with the purposes of later comparing the German and the Swedish

methods for diagnosing and quantifying cataract.

National collaboration

We have also started discussion with a research group in Gothenburg who are studying cancer risks in a large cohort of infants treated for hemangioma in childhood. A first joint planning meeting will take place in October 1993, with the purpose of sharing experience and discussing possibilities of pooling the data for future analyses.

Head of project 6: Prof. A. Becciolini

Scientific staff

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II. Objectives for the reporting period

The work performed dealt chiefly with enlarging the size of the cohort of patients affected by breast cancer and treated with ionizing radiation, chemotherapy or hormonotherapy after surgery. The cohort now consists of 4635 patients (previously 1449) treated from January 1965 to December 1991 (contributing 30233 person-years). A preliminary analysis of 2100 patients affected by breast cancer and treated with radiotherapy only following surgery at the Radiotherapy Centre of the University of Pisa has been performed.

III. Progress achieved including publications

Work during the reporting period concerned the analysis of clinical records of breast cancer patients, with an extension in the number of patients (4635), collected from January 1965 to December 1991. Treatment policy changed during that time. Before 1977, patients were submitted to radical mastectomy and radiotherapy; from 1980 the treatments were generally conservative surgery and radiotherapy. Irradiation to lymphatic drainages namely mammary chains, supraclavicular region, axillae ± chest wall was routinely used in all patients with involved axillary lymphnodes.

The age of patients ranged from 22 to 92 years (mean 55, median 54). The irradiation field included the breast (33% of patients), lymphatic drainages (9%), chest wall plus lymphatic drainages (6%). No irradiation was given to 52% of patients: 15% were submitted to chemotherapy and 22% to hormonotherapy. Radiation dose ranged from 44 to 60 Gy. Some patients received both radiotherapy and either chemotherapy or hormonotherapy.

The histopathology of the second tumour has been always verified: it must have a clear malignant histologic pattern, not be a metastasis and must be distinguished without reasonable doubt from the first cancer.

In the total series of 4635 patients (contributing 30233 person-years), 1.5% of cases showed a synchronous breast cancer (which appeared within 1 year), 3% a metachronous breast cancer and in 2% of cases a primary tumour in a different site was observed.

The mean follow up was 6.75 years (median 5.33), with the 25th percentile being 2.75 and the 75th percentile being 9.67 years. The mean detection time of the second primary tumour was 5.33 years, median 4.5. The more frequent locations of second tumour were: breast (158 cases), colon-rectum (23), stomach (18), endometrium (10), leukaemia (7), ovary (6), kidney or uterine cervix (5), lung, anus or non-Hodgkin lymphoma (3), thymus (2) and , in 1 case, plasmocytoma, larynx, carcinoid, bladder, liposarcoma, biliary duct, oesophagus, phaeochromocytoma, tongue, basaloma, epithelioma.

Table 1 shows the frequency of second primary cancers according to the type of treatment given for the initial cancer.

Table 1

	LEUKAEMIA	METACHRONOUS BREAST CANCER	OTHER CANCER	ALL CANCERS	No. PTS.
RT BREAST	2	38	22	77	1511
RT NODES ± CHEST WALL	4	50	20	86	710
NO RT	1	70	51	168	2414
Total	7	158	93	331	4635

In evaluating the incidence of second cancers, comparison has been made with expected values obtained from the Tuscany Cancer Registry (1985-1987).

The Standardized Incidence Ratios (SIRs) (observed/expected) were 1.51 for leukemia (not significant, n.s.) based on all patients and 4.72 ($p<0.05$) in patients treated with radiotherapy. For metachronous breast cancer the corresponding SIRs were respectively 3.07 ($p<0.05$) and 5.00 ($p<0.05$); and for other cancers 0.54 ($p<0.05$) and 0.81 (n.s.). The SIR for all cancers was 1.40 ($p<0.05$) in the group of patients treated by radiotherapy.

The incidence of controlateral breast cancer seems to be influenced by previous breast cancer (persistence of risk factors), adjuvant chemo-hormonotherapy, and radiotherapy. Non-statistically significant differences in risk are observed as a function of age, whereas a borderline difference ($p=0.052$) was observed in patients treated on local or broad fields. The rate ratio in patients treated only by radiotherapy is 1.44 for a latency of 7-15 years (n.s.) and 2.41 ($p<0.05$) for latency >15 years.

The analysis of leukaemia incidence in patients treated by radiotherapy only shows that the rate ratio is 5.7 for local irradiation and 11.2 for broad fields ($p<0.05$). The detection time ranged from 3.3 to 7.7 years for acute myeloid or lymphatic leukemia, whereas one case of chronic lymphatic leukemia appeared after 13.3 years. A case of chronic myeloid leukemia after 7.7 years was observed in the group of non-irradiated patients.

Unlike Florence, where most patients returned periodically during the follow-up for clinical checks, patients at other Centres were often lost some time after treatment. It is therefore necessary to collect information on them from town Councils or from community physicians. For this reason, the analysis of the incidence of second primary cancers is longer and requires more resources at the other Centres involved in the study.

At the Institute of Radiotherapy of the University of Pisa the data on second primary tumours after treatment for breast cancer are still being collected. In a preliminary analysis 29 second metachronous tumours have been identified (17 controlateral breast cancer, 3 uterus, 2 colon-rectum, and 1 each of liver, ovary, vagina, oesophagus, thyroid, lung, tonsil). Three tumours (2 uterus and 1 controlateral breast cancer) appeared within the first year. The doses ranged from 30 to 61 Gy and most patients received 2 Gy/day for 5 days/week up to a total of 48 Gy. The radiation sources were 60Co and linear accelerator. An accurate update of the follow-up is continuing.

A secondary part of the study deals with the determination of some biochemical parameters in blood of patients during radiotherapic treatment and follow up. Molecules such as polyamines and tumor antigens have been assayed in some patients at risk as markers for early detection of cancer.

Publications

A. Becciolini, P. Pacini, A. Biggeri, P. Olmi et al. Valutazione dell' incidenza di secondi tumori in soggetti sottoposti a radioterapia. Atti Congresso Nazionale Associazione Italiana Radioprotezione medica, Roma, 30 giugno-2 luglio 1993, in press.

P. Pacini, A. Becciolini, A. Biggeri et al. Incidence of a second primary tumor in patients treated for breast cancer. In preparation.

A. Becciolini, S. Porciani, A. Lanini. Polyamines. In: "Updating on tumour markers in tissue and in biological fluids: basic aspects and clinical applications". A. Ballesta, G.C. Torre, E. Bombardieri, M. Gion, R. Molina, Eds., Minerva Medica, Torino, 413-434, 1993.

A. Becciolini, S. Porciani, A. Lanini, M. Tommasi, P. Olmi, A. Chiavacci. Prognostic significance of Tissue Polypeptide Antigen (TPA) in the head and neck carcinoma. Acta. Oncologica 32, 295-299, 1993.

IV. Objectives for the next reporting period

In Florence, the analysis of clinical records of patients treated with ionizing radiation for cancers of head and neck, endometrium and uterine cervix will be extended relative to the previously reported analyses (1960-1984 for head and neck and 1977-1985 for gynaecological cancers) to December 1991. Another part of the study deals with the revision and the extension of a cohort of patients (more than 600) treated with iodine-131 for thyroid cancer, from 1977 to December 1991, at the Nuclear Medicine Section. During the next period the analysis of data from Pisa and Modena will continue.

Head of project 7 : Dr. S. RICHARDSON

Project : *Statistical methods, biases and interpretation of geographical correlation studies with application in analysing the geographical association of cancer and radiation exposure*

II - Objectives for the reporting period (1/7/92 - 31/8/93)

a) Set up the data file to analyse the geographical distribution of childhood leukemia in Great-Britain in relation to natural radiation exposure.

b) Assess the performance of the modified tests of association (previously developed under normality assumptions) in the context of small area statistics including within-area Poisson variability.

c) Investigate sources of biases in geographical studies, with regards to the joint effect of risk factors at a group level. Quantify the biases using a simulation model in the case of multivariate normal regressions.

Objectives for the next period

a) Compare statistical methods for assessing covariate influence on disease incidence with reference to the analysis of childhood leukemia and radiation exposure in Great Britain.

b) Further quantify biases in geographical studies in the case of Poisson regressions.

III - Progress achieved including publications

a) Geographical distribution of childhood cancer in Great Britain

This is part of a collaborative project with C. Muirhead (NRPB) and G. Draper (CCRG).

We have transferred a data file from NRPB and CCRG containing the cases of leukemia and other childhood cancer between 1969 -1983, mean indoor radon concentrations, indoor and outdoor gamma dose rates and socio-demographic variables for 459 county districts in England and Wales. A geographical data file has been set up containing the geographical coordinates and a nearest neighbourhood structure for each county.

In the next part of the project, this data file will be analysed by different statistical methods which take into account the spatial structure.

b) Modified tests of association

In a previous contract (BI6 126 F), modified tests of simple and partial correlations for spatially autocorrelated variables were designed, based on an underlying assumption of normality of the variables. In the first part of this contract, we have tested the performance of these tests of association in a case which includes within-area Poisson variability.

The specific model was the following :

- we considered a region composed of N areas, indexed by i ;
- for each area, let O_i be the observed number of cases, E_i the expected number of cases (age adjusted) and t_i , the unknown relative risk for area i. The ratio O_i / E_i is the Standardised Mortality Ratio, SMR_i. We made the usual hypothesis for small areas that O_i is distributed as a Poisson variable with mean $E_i\, t_i$. The spatial structure in $\underline{t} = \{t_i, 1 \leq i \leq N\}$ and in the covariate $\underline{z} = \{z_i, 1 \leq i \leq N\}$ was introduced by supposing that $\log \underline{t}$ and $\underline{z}$ follow multivariate normal distribution with spatially autocorrelated covariance matrix indexed by autocorrelation parameters ρ_t, ρ_z.

The correlation r between $\{\log SMR_i\}$ and $\{z_i\}$ was tested

- by the classical test : t_N with $\sqrt{N-2}\ \ r\ (1- r^2)^{-1/2}$ following a t distribution with N-2 d.f. (no allowance for spatial dependence)

- by the modified test $t_{\hat{M}}$ where $\hat{M}$ represents effective degrees of freedom taking into account the spatial structure (Richardson, Statistics in Medicine, 9, 515-528, 1990).

Simulations were performed to assess the type I errors under the null hypothesis H_0 of independence between $\underline{t}$ and $\underline{z}$, and for several values of the autocorrelation ρ_t and ρ_z.

A summary of the results is presented in Table 1, with the Gaussian case given as a reference. In the Poisson case, one can see that even for low autocorrelation in $\underline{t}$ or $\underline{z}$, the type I error of the modified tests is inflated with respect to its nominal value of 5%. Nevertheless, the over-significance of the $t_{\hat{M}}$ test stays moderate and the modified test represents still a considerable improvement in comparison to the standard t_N test of correlation.

Table 1 : Type I errors of the modified test, 5% nominal level.

Autocorrelation $\rho_t = \rho_z$		0	0.2	0.4	0.6	0.8
Gaussian case (no Poisson fluctuation)	t_N	**5.1** (3.7 - 6.5)	**6.5** (5.0 - 8.0)	**8.5** (6.8 - 10.2)	**16.5** (14.2 - 18.8)	**37.0** (34.0 - 40.0)
	$\hat{t}_M$	**5.3** (3.9 - 6.7)	**5.4** (4.0 - 6.8)	**5.1** (3.7 - 6.5)	**5.7** (4.3 - 7.1)	**4.5** (3.2 - 5.8)
Poisson case Oi ~ P (Ei Yi)	t_N	**7.6** (6.0 - 9.2)	**8.5** (6.8 - 10.2)	**10.5** (8.6 - 12.4)	**15.5** (13.3 - 17.8	**36.5** (33.5 - 39.5)
	$\hat{t}_M$	**7.4** (5.8 - 9.0)	**8.8** (7.0 - 10.6)	**11.3** (9.3 - 13.3)	**13.0** (10.9 - 15.1)	**10.4** (8.5 - 12;3)

c) Ecological biases with 2 risk factors

We considered the following situation. Let X and Y be 2 dichotomous risk factors with marginal prevalence x and y in an area, R_X (R_Y), the rate ratio for *sole* exposure to X (resp. to Y) and R_{XY} the rate ratio for *joint* exposure to X and Y with corresponding joint prevalence z.The rate for this area is :

$$I(x,y,z) = I_0 \{1+x (R_X - 1) + y(R_Y - 1) + z(1 - R_X - R_Y + R_{XY})\} \quad (1)$$

where I_0 is the baseline rate for the unexposed. In general joint exposure prevalence z is not known for each area and to estimate R_X and R_Y, further assumptions are necessary.

- *If we suppose that the joint effect of X and Y is additive* : $R_{XY} = R_X + R_Y - 1$ then it is sufficient to regress simply on x and y with the reduced model :

$$I(x,y) = I_0 \{1 + x (R_X - 1) + y (R_Y - 1)\} \quad (2).$$

- *If we suppose independence between the distributions of X and Y within the group*, i.e. z = xy : then one should introduce a term xy in the regression.

$$I(x,y) = I_0\{1 + x (R_X - 1) + y (R_Y - 1) + xy (1 - R_X - R_Y + R_{XY})\} \quad (3).$$

Ecological bias

A simulation model was set up to assess the consequences of using inappropriately equations (2) or (3) ; in particular

- misspecification of the regression model with respect to the underlying Relative Risk model,
- use of xy as a joint prevalence even when the exposures are not independent.

Simulation set up

We considered 50 geographical units with marginal prevalences x_i and y_i, i = 1,... 50. The geographical association between X and Y *accross* the 50 units is characterised by a parameter δ. The within-unit association between X and Y is specified by an odds ratio ψ (the same for all units). The joint effect of the risk factors followed either an additive risk model or a multiplicative risk model ($R_{XY} = R_X R_Y$). The incidence rate for each unit was generated by using equation (1) plus a normally distributed random error term, where the ratio of within-unit variance to the between-units variance can be controlled by the variance of the random error term. Each set up was simulated 100 times and estimation was carried out using equations (2) or (3) and a multivariate normal setting.

Results

First the case of independent risk factors within unit (z=xy) was investigated (Table 2). When the regression model correspond to the underlying risk models, estimation of the relative risks is correct. Misspecification is assessed on the off-diagonal elements of Table 2. The results are clearly asymetric : when the risk model is multiplicative, using the regression model (2) leads to *overestimation* of the RR, whereas when the risk model is additive, using the regression model (3) still produces good estimates with slightly increased variances.

These results were confirmed for different values of the geographical association δ, and for other values of R_X and R_Y.

Table 2 : True relative risks : $R_X = R_Y = 2$, $z = xy$ ($\psi = 1$)

	Risk model	
Regression models	Additive	Multiplicative
$b_0 + b_1x + b_2y$ (2)	$\hat{R}_X$ = 2.01 (0.01) $\hat{R}_Y$ = 2.01 (0.01)	$\hat{R}_X$ = 2.97 (0.02) $\hat{R}_Y$ = 2.97 (0.02)
$b_0 + b_1x + b_2y + b_3xy$ (3)	$\hat{R}_X$ = 1.99 (0.02) $\hat{R}_Y$ = 1.99 (0.02)	$\hat{R}_X$ = 1.98 (0.04) $\hat{R}_Y$ = 1.98 (0.04)

[within-unit s.d./between-unit s.d. = 1/4]

When the within-unit fluctuations is increased and becomes of the same order as the geographical variance, regression with equation (3) in the case of the multiplicative model leads to relative risks estimates which are somewhat biased downwards, but the degree of bias is less severe than that encountered if regression model (2) is used.

Finally the performance of ecological regressions give by equation (3) when the true joint exposure prevalence $z \neq xy$ (equivalently $\psi \neq 1$) was assessed (Table 3). As in Table 2, regression equation (2) in the case of a multiplicative risk model leads to an *overestimation* of the RR. We note a good performance of regression equation (3) for moderate within-unit association between the risk factors ($2 \leq \psi \leq 3$). When the within-unit association between the risk factors is strong (ψ=4), regression equation (3) leads to slight overestimation with increased standard error.

Table 3 : True relative risks : $R_X = R_Y = 2$

	Multiplicative risk model		
Regression models	$\psi = 2$	$\psi = 3$	$\psi = 4$
$b_0 + b_1x + b_2y$ (2)	$\hat{R}_X$ = 2.90 (0.02) $\hat{R}_Y$ = 2.85 (0.02)	$\hat{R}_X$ = 3.11 (0.03) $\hat{R}_Y$ = 3.11 (0.03)	$\hat{R}_X$ = 3.54 (0.04) $\hat{R}_Y$ = 3.55 (0.04)
$b_0 + b_1x + b_2y + b_3xy$ (3)	$\hat{R}_X$ = 2.09 (0.04) $\hat{R}_Y$ = 2.05 (0.04)	$\hat{R}_X$ = 2.04 (0.05) $\hat{R}_Y$ = 2.05 (0.06)	$\hat{R}_X$ = 2.32 (0.08) $\hat{R}_Y$ = 2.30 (0.18)

[within-unit s.d./between-unit s.d. = 1/4]

The good performance of regression equation (3) when $1 \leq \psi \leq 3$ was checked for different values of the geographical association δ and for other values of R_X and R_Y. As previously, we found that in this multivariate normal setting, a key factor for a correct estimation of the relative risks using equation (3), is that the between-unit variance should be large with respect to the within-unit variance.

In the next part of the project, this aspect will be investigated in depth using Poisson regression models.

Related publication :

S. Richardson, C. Guihenneuc, V. Lasserre. Spatial linear models with autocorrelated error structure. The Statistician, 41, 539-557 (1992).

Head of project 8: Dr. Hill

II. Objectives for the reporting period

1) - To study mortality in the population aged 0 to 24, residing around 6 French nuclear sites in operation before 1976.

2) - To study the incidence of thyroid cancer in the vicinity of Chooz, a nuclear plant which is in an area covered by a thyroid cancer registry.

Objectives for the next reporting period

1) - To extend the leukaemia mortality study around nuclear sites to all 13 installations operating before 1985, including all deaths occurring between 1968 and 1989 in the population aged 0 to 24.

2) - To study leukaemia mortality among children living in French towns with a recent and large increase in population.

III Progress achieved including publications

1) Study of mortality around nuclear sites.

The main sites in operation during 1975 or before were selected for inclusion in the study, in order to have a minimum follow-up of 10 years for mortality data. Table 1 shows the six sites selected.

Table 2 includes, for each cause of death considered, the observed and expected number of deaths (from national statistics) in the exposed communes, and the standardised mortality ratio. The total number of person-years is also given.

Three thousand and sixty four deaths were observed, which is very close to the expected number from national mortality statistics. Fifty eight leukaemia deaths were observed around nuclear sites, which is slightly less than the 67 expected from national mortality. Among the other causes of death considered, an excess of deaths from Hodgkin's disease (SMR=197), and a deficit of death from malignant brain tumor (SMR=41) were observed. These two differences are not statistically significant after correcting for the number of tests performed.

There is no significant increase in the risk of death with a decreasing distance to the nuclear site, except for overall mortality (p=0.002).

For each cause of death, heterogeneity between installations was tested. A heterogeneity between installations was observed for overall mortality ($p=10^{-4}$) and for lung cancer mortality (2 deaths around Chinon, p=0.02). For Hodgkin's disease, 8 of the 12 observed deaths occurred in the vicinity of Marcoule, but the test for heterogeneity between installations was not statistically significant (p=0.22).

Table 1 : Nuclear installations included in the study

Name of site, type of activity* name of company	Type of installa-tion +	Name of the reactors	Date of first operation
Chinon, PE, EDF	GGR	Chinon A1-3	16-09-62
Chooz, PE, EDF	PWR	Chooz A1	18-10-66
La Hague,R, COGEMA	R	La Hague	1968
Marcoule, PE+R, COGEMA	GGR+FBR R	Marcoule G1-3 Phénix	09-56
St Laurent, PE,EDF	GGR	St Laurent A1-2	07-01-69
St Vulbas, PE, EDF	GGR	Bugey 1	07-12-71

* PE : Production of electricity
R : Reprocessing
EDF : Electricité de France,
\+ PWR : Pressurised Water Reactor
GGR : Graphite-Gaz Reactor
FBR : Fast Breeder Reactor

Sources : two anonymous documents from Commissariat à l'Energie Atomique.

Table 2 : Observed (O) and expected (E) number of deaths, and Standardised mortality ratio (SMR) in exposed communes *

Cause of death	O	E	SMR (%)
Overall mortality	3 064	3 093.7	99
All malignant tumors	166	171.7	97
Malignant brain tumor	6	14.5	41
Brain tumor of unspecified type	16	14.1	113
Lung cancer	2	2.1	94
Hodgkin's disease	12	6.1	197
Non Hodgkin lymphoma	16	15.5	103
Myeloma	0	0.3	0
Leukaemia	58	66.9	87
Lymphoid leukaemia	13	19.0	68
Person-years	2 892 000		

* All SMR's are not statistically different from 1

2) Study of thyroid cancer incidence in the vicinity of Chooz

The nuclear power plant at Chooz, north Ardennes, is one of the oldest in France and was in operation between October 1966 and December 1991. Data on new cases of differentiated thyroid carcinomas diagnosed between 1979 and 1991 in the population residing around the plant were obtained from the Champagne-Ardennes Thyroid Cancer Registry. The registry covers the two "départements" of Marne and Ardennes. All the "communes" within 40 km of Chooz nuclear power plant were included in the study. The rest of the registry catchment area (>40 km from the plant) was considered the reference population.

During the period 1979 to 1991, 77 cases of differentiated thyroid carcinoma were diagnosed in the vicinity of Chooz (63 in women and 14 in men).

Table 3 gives the size of the population at risk, the observed and expected number of cases, and the SIR according to distance to the plant. No significant increase was observed in the SIRs near Chooz (test for trend : p=0.9). This result was confirmed when the exact distance of each commune was used in a log linear model of the SIRs.

Table 3 : Number of person-years, observed and expected number of differentiated thyroid incident cases, by geographical zone.

Distance from plant (km)	Number of communes	Person-years (in thousands)	Number of differentiated thyroid cancer		SIR (%)* [95%CI]
			Observed (O)	Expected (E)	
0-9	14	224	9	8.02	112 [58-196]
10-19	3	104	8	3.65	218 [109-396]
20-29	16	393	10	14.25	70 [38-119]
30-39	38	1 131	50	41.67	120 [94-152]
Reference population	1010	9 199	340	..	..

* 100 O/E. SIR : Standardised Incidence Ratio.

Hill C, Laplanche A. Mortalité autour d'installations nucléaires françaises. Paris: INSERM 1992.

Rekacewicz C, de Vathaire F, Delisle MJ. Differentiated thyroid carcinoma incidence around the French nuclear plan in Chooz. Lancet 1993;341: 493.

Head of project 9: Dr. de VATHAIRE

II. Objectives for the reporting period

To conduct a cohort study of second cancer incidence amongst patients treated for cancer during childhood .

III. Progress achieved including publications

The inclusion of the children in the cohort is ongoing and the analysis has not yet begun. Hence, the following results are preliminary; no results concerning the cohort study have yet been published.

3606 children are currently included in the study, amongst whom 131 have developed at least one second cancer. The mean age at first cancer diagnosis was 6 years (range 0-17). A total of 137 second cancers have been reported (Table 1). The most numerous type of first cancers were Wilm's tumour (731) and lymphomas (712). 70% of the children had received chemotherapy and 76% radiotherapy.

The highest values for the 30 years cumulative risk of second cancer were observed after bone sarcoma (29%) and neuroblastoma (26%) (Table 2). The 30 years cumulative risk of second cancer was only 1% if children were treated by surgery alone, but 10% after radiotherapy alone, 21% after chemotherapy alone, and 20% after both radiotherapy and chemotherapy (Table 3). No effect of sex was observed (Table 3). The difference between the two countries (Table 3) may be explained by difference in treatments (p-value for country effect when adjusting for treatment and first cancer type : p=0.3).

The most numerous type of second cancer were bone sarcoma (35), soft tissue sarcoma (14), thyroid carcinoma (13), female breast cancer (12), and leukaemia (12). The 30 years cumulative risk of female breast cancer was particulary high (4%); see Table 4.

Table 1 - Children by centre

Centre	No. children	No. children with a second cancer
Institut Gustave Roussy (Villejuif, Fr)	2137	96
Institut Curie (Paris, Fr)	307	7
Royal Marsden Hospital (GB)	239	4
Great Ormond Street Hospital (GB)	420	9
St Bartholomew's Hospital (GB)	203	8
Centre Lacassagne (Nice, Fr)	19	0
Institut Jean Godinot (Reims ,FR)	88	2
Centre Claudius Régaud (Toulouse, Fr)	193	5
Total	3606	131

Table 2 - Treatments received by the 3606 children

First cancer	No. children	Chemo-therapy	Radio-therapy	Children with second cancer	30 years cumulative risk of second cancer (sd)
Wilm's tumour	731	85%	77%	20	9% (3%)
Neurobastoma	482	70%	61%	14	26% (9%)
Lymphoma	712	82%	83%	24	16% (5%)
Soft tissues	528	71%	70%	29	14% (4%)
Bone	242	73%	83%	15	29% (11%)
Brain	419	36%	98%	13	9% (3%)
Gonadal	125	54%	45%	4	6% (4%)
Thyroid	34	3%	44%	1	14% (13%)
Retinoblastoma	129	61%	84%	5	8% (4%)
Others	207	58%	70%	6	10% (5%)
Total	3606	70%	76%	131	14% (2%)

Table 3 - Second cancer incidence according to treatment, sex and country

	No. patients with second cancer	30 years cumulative risk of second cancer (s.d.)
Treatment		
No chemotherapy, no radiotherapy (259)	2	1% (1%)
Chemotherapy alone (602)	9	21% (10%)
Radiotherapy alone (838)	34	10% (2%)
Chemo+radiotherapy (1907)	86	20% (5%)
Sex		
Male (2030)	66	12% (2%)
Female (1574)	65	15% (3%)
Country of treatment		
Great Britain (862)	21	8% (3%)
France (2744)	110	16% (2%)

Table 4 - Sites and general characteristics of the 137 second primary cancers

Site of the second cancer	Delay (mean)	Age (mean)	Surgery	Chem.	Rad.	Chemo + Rad.	30 years cumulative risk of second cancer
Bone (35)	9	15	0	0	2	33	2% (1%)
Soft tissue (14)	12	20	0	1	2	12	2% (1%)
Breast (12)	20	29	1	2	5	4	4% (2%)
Leukaemia (12)	9	14	0	2	2	8	2% (1%)
Thyroid (13)	16	22	0	0	6	7	2% (1%)
Brain (10)	12	16	0	1	5	4	2% (1%)
Melanoma (7)	11	20	0	1	1	5	0.8% (0.4%)
Other skin (12)	16	23	0	0	6	6	2% (1%)
Digestive (5)	17	23	0	1	0	4	1% (1%)
Respiratory (1)	4	5	0	1	0	1	0.03% (0.03%)
Others (15)	16	20	1	2	4	8	1% (1%)

Table 5 - Sites of the 137 second primary cancers according to that of the first cancer

		Second cancer								
First	All	Bone	Soft tissue	Breast	Leuk-aemia	Thy-roid	Brain	Mela-noma	Other skin	Other
Wilm's tumour (731)	20	1	4	1	4	2	1	2	2	3
Neurobastoma (482)	14	1	1	2	2	4	1	-	3	-
Lymphoma (712)	24	6	1	4	3	3	2	-	-	5
Soft tissue (528)	32	10	4	2	1	2	2	2	4	5
Bone (242)	15	11	2	-	1	1	-	-	-	-
Brain (419)	15	1	1	1	1	1	4	2	2	2
Gonadal (125)	5	-	1	1	-	-	-	1	-	2
Thyroid (34)	1	-	-	-	-	-	-	-	1	-
Retinoblastoma (129)	5	4	-	-	-	-	-	-	-	1
Others (207)	6	1	1	1	-	-	-	-	-	3
All sites	137	35	15	12	12	13	10	7	12	21

IV. Objectives for the next reporting period

Plan for September 1993- 31 May 1994 (end of original contract)

- To extend the cohort study in order to include about 5000 patients treated for a first cancer during childhood.

Plan for 1 June 1994- 30 June 1995 (extension of contract)

- To finish the dosimetry of the study
- To improve the model of dose estimation in order to estimate doses for children treated with electrons.
- To begin the analysis of the study.

Head of project 10: Dr. R.R. Wick

This project deals with about 1500 ankylosing spondylitis patients treated between 1948 and 1975 with relatively low doses of radium-224, injected intravenously. These doses were far lower than those to the earlier patients in the original Spiess cohort (see project 11).

II. Objectives for the reporting period

Contact and follow-up of patients of the exposure group and the control group. Registration of causes of death. Comparison of the results from the exposure and control groups with respect to the risk of late effects in bone, haematopoietic tissue, and other organs known or supposed from project 11 to be related to the ^{224}Radium treatment.

III. Progress achieved including publications

In an epidemiological study of the somatic late effects risk following incorporation of a short lived α-emitter, 1473 ankylosing spondylitis patients treated with repeated intravenous injections of ^{224}Ra in the years 1948-75, have been observed by GSF (Table 1). The usual therapeutic plan consisted of a total of 10-12 injections of 1.036 MBq (28 μCi) of ^{224}Ra each, given at weekly intervals; this would result in a cumulative α-dose of 0.56 - 0.67 Gy to the marrow-free skeleton of a 70-kg-man (standard man). These patients have been followed together with a control group of ankylosing spondylitis patients not treated with radioactive drugs and/or X-rays.

Up until May 1993 (mean follow-up time 19.9 yr), 595 patients in the exposed group and 722 patients in the control group had died. Causes of death have been ascertained for 578 and 668 patients respectively (Table 2). The remaining cases are still being investigated.

Table 3 shows the types of tumours occurring in our patient groups compared to the cases expected from the three regional tumour registries of the FRG, namely those of the Saarland, of Hamburg and of the former GDR. The expected cases shown there are the ranges resulting from calculations from these three registries, as there is no Federal cancer registry for the whole FRG. In Table 3 we restricted our interest to those diseases which are known or implied from project 11, the higher dose study, to be associated with prior administration of ^{224}Ra. However, in contrast to the findings in the high dose group, there are no significant differences between the observed and expected cases for cancers of stomach, liver, urinary system, or the female breast, neither for the exposed nor for the control group. Furthermore, in addition to the four cases of malignant bone tumours (according to the Histological Typing of Bone Tumours of the WHO) which have been reported earlier, we observed 10 cases of leukaemia in the exposed group (vs. 2.7-2.8 cases expected, $p < 0.001$) and 6 cases in the control group (vs. 3.3 - 3.5 exp., $p = 0.14$). Subclassification of the leukaemias shows a clear preference for chronic myeloid leukaemia (CML) in the exposed group (4 cases obs. vs. 0.8 cases exp., $p = 0.009$), whereas in the control group the observed cases of CML are within the range of expectancy. Similar observations have not been made in the patients of project 11 (Spiess et al), who have been treated at higher doses and dose rates.

This increased incidence of leukaemias in our exposed group is in line with results from animal experiments with bone seeking α-emitters given at low dose rates. The induction of myeloid leukaemia in mice has there been demonstrated down to dose rates of only a few mGy/day, not only for ^{224}Ra, but also for ^{239}Pu, an α-emitter which, like ^{224}Ra, deposits preferentially on the bone surface.

Table 1. Follow-up status and exposure parameters for the ankylosing spondylitis patients

	Exposed Group	Control Group
Total number of patients	1579	1464
Treated with X-rays additionally	106	126
Remaining patients	1473	1338
Deceased patients	595	722
Cause of death certified	578	668
Cause of death not yet known, still being investigated	17	54
Mean injected amount of ^{224}Ra (MBq/kg)	0.17	-
Mean α-dose to the skeleton (Gy)	0.67	-
Mean injection period (weeks)	10.4	-
Mean follow-up time (years)	19.9	20.4

Table 2. Tumours of bone and haematopoietic tissue after treatment with ^{224}Radium

	Exposed Group	Control Group
Deceased patients, cause of death certified	578	668
Total tumours	128 (23*)	148 (13)
TUMOURS OF BONE AND BONE MARROW	5	2
Exostosis (osteochrondroma)	1 (1)	1
Malignant tumours of bone and bone marrow	4	1
Fibrosarcoma	1	0
Fibrous histiocytoma (spindel cell sarcoma)	1	0
Reculum cell sarcoma	1	0
Medullary plasmocytoma	1	1
MYELOPROLIFERATIVE DISEASES	7 (1)	3
Myeloid leukaemia	6	3
Chronic myeloid leukaemia	4	1
Acute myeloid leukaemia	2	2
Osteomyelosclerosis	1 (1)	0
Lymphatic leukaemia	3	2
Leukaemia of unknown type	1	1

* living

Table 3. Observed tumours and cases obs/exp

	Exposure Group		Control Group	
	obs.	exp.	obs.	exp.
Total number of tumours	118	118-144	148	154-188
Stomach	16	11.9-14.1	15	16.0-19.1
Liver	1	2.5-3.7	7	3.4-4.8
Urinary system	9	8.1-10.6	10	10.7-13.9
Female breast	1	2.6-4.3	1	2.2-3.6
Non-Hodgkin lymphoma	2	0.9-1.8	1	1.0-2.3
Hodgkin's disease	1	0.8-1.1	0	0.8-1.1
Skeleton	4	0.7-2.4	1	0.8-2.8
Leukaemias	10	2.7-2.8	6	3.3-3.5
Myeloid leukaemia	6	(1.5)	3	(1.9)
Chronic myeloid leukaemia	4	(0.8)	1	(1.1)
Acute myeloid leukaemia	2	(0.7)	2	(0.8)
Lymphatic leukaemia	3	(1.3)	2	(1.6)
Leukaemia of unknown type	1		1	

Publications

R.R. Wick, W. Gössner. History and current uses of ^{224}Ra in ankylosing spondylitis and other diseases. Environ. Intern. (1993), in press.

R.R. Wick, D. Chmelevsky, W. Gössner. Mortality from chronic myeloid leukaemia and other diseases in Ra-224 treated ankylosing spondylitis patients. European Society for Radiation Biology, 24th Annual Meeting, Erfurt (Germany), October 4-8, 1992, Book of Abstracts p.153.

Head of project 11: Prof. Dr. Heinz Spiess

This project is concerned with the early patients who were subject to high doses of radium-224 in a German hospital shortly after World War II, as juveniles for bone tuberculosis, and as adults for ankylosing spondylitis.

II. Objectives for the reporting period:

- To review existing data. To determine stochastic and non-stochastic radiation effects and how their risks depend upon dose, time and age.

- To send standard questionnaires to women treated as juveniles, to get information about their state of health and the appearance of breast cancers.

- To send questionnaires to all living patients who were treated with Ra-224 as adults to continue their follow-up.

- To cooperate with Prof. F. Stefani at the Clinic of Ophthalmology of the University of Munich and with Prof. Breipohl/Prof. Hockwin at the Institute of Experimental Ophthalmology of Bonn University who specialise in the Scheimpflug camera (see project 12).

- To visit patients treated as juveniles (living in northern Germany) to check for cataracts using a Scheimpflug camera.

- To set up a control group of tuberculosis patients of the years 1949-1953, not treated with Ra-224, and drawn from a file of a specialised lung clinic.

III. Progress achieved including publications:

At 3-year intervals we are following the health of 900 patients (509 adult males, 173 adult females, 111 boys, and 107 girls) who received repeated injections of Ra-224 after World War II, for treatment of ankylosing spondylitis or tuberculosis, but also for other non-cancerous diseases.

On an average, the dosage in kBq/kg was about twice as high to the children as to the adults, and because of the growing skeleton, the average skeletal dose was much higher in the children than in the adults. Usually the patients received 1 or 2 injections per week. The average length of treatment was 6 months for the adults and 11 months for the juveniles. At the time of last contact (June 1993), 310 of the 900 patients were still alive.

We observed 56 cases of bone sarcomas; 2 patients had two bone sarcomas. The last bone tumour appeared four years ago.

There were 125 soft tissue malignancies observed. This exceeds the expected number based on the distribution in age, the length of the follow-up of the cohort, and the age-specific population rates for the different malignancies. The age-specific rates were taken from the Saarland tumour registry.

The breast cancer excess came as a surprise, with 22 cases observed versus approx. 6.5 cases expected. All but one case occurred more than 10 years after irradiation and all but one occurred after the age of 35. Among those patients who were treated as adults, the 11 observed cases represent about a twofold increase compared with the approx. 5.1 expected cases.

Furthermore, for those patients given RA-224 as juveniles, an eightfold increase occurred (11 cases observed vs. 1.4 expected, $p<0.000001$).

Liver cancers occurred in seven Ra-224 patients, ie. in a significantly larger number than the 2.4 cases expected ($p=0.01$).

Kidney cancers have occurred in six patients compared with 1.9 expected ($p=0.01$). With one exception, all of these cancers were "hypernephromas", the most common form of kidney cancer. The tumour appearance times ranged from 12 to 35 years after the start of Ra-224 injections.

There were 12 observed cases of prostate cancer vs. 5.6 expected ($p=0.01$) and 8 observed cases of bladder cancer vs. 3.8 expected ($p=0.04$).

Leukaemia occurred in six patients compared with two expected cases based on German Cancer Statistics. The enhanced leukaemia rates observed in the control group of project 10 would correspond to five leukaemia cases among the Ra-224 spondylitis patients, whereas 2.2 cases would be expected according to German population rates.

Four of the six leukaemias within our study occurred among the 396 spondylitis patients; only two occurred among the 504 other patients who mostly had tuberculosis. Only one of the "juvenile" patients has developed leukaemia; this female tuberculosis patient had chronic lymphatic leukaemia, a type not assumed to be associated with radiation exposure.

In the continued follow-up, the possibility of increased rates of additonal types of cancers will need to be monitored.

To avoid confusion with age-related cataracts, that part of our project dealing with cataracts is based on the 218 "juvenile" patients in the Spiess series who had been injected with Ra-224 at age 20 or younger. Of these patients, 90 are known to have died. The ophthalmologists' addresses were known for 92 patients, and 65 ophthalmologists responded to a questionnaire asking for data on the visual acuity, causes of visual disturbances, intraocular inflammations, degenerations, retinal dystrophies or intraocular tumours, ophthalmic medication, earlier eye surgery and data on lens opacities classified by slitlamp examination. Thirty-five out of 60 invited "juvenile" patients have been visited to examine their lenses with the Scheimpflug camera. For results see publication (1).

To find out whether or not the increased number of breast cancers in the cohort represents an effect of radiation exposure, a control group of tuberculosis patients of the years 1949-1953, not treated with Ra-224, and drawn from a file of a specialised lung clinic, has been set up and is now being examined using the same procedure as for the exposed group. In order to get in contact with these patients, we asked for their latest addresses at different registration offices. Meanwhile, standard questionnaires have been completed by many of the patients. For more detailed results see Table 2.

Publications

(1) J.P. Scharff, E. Nekolla, P. Egner, H. Roos, A. Wegener, A.M. Kellerer, H. Spiess, F.H. Stefani. Findings in human lenses 40 years after injection of Ra-224. Accepted for publication in Ophthalmic Research (1993).

Table 1. Diseases of the Radium-224 patients (June 1993)

Disease	Children and juveniles	Adults	Total
1. Skeletal diseases			
Bone sarcoma	38	18	56
Exostosis	29	0	29
Growth retardation	28	0	28
Tooth breakage	40	20	60
2. Soft tissue diseases			
Cataract	28	61	89
Liver (non-cancer)	4	35	39
Kidney (non-cancer)	11	69	80
Diabetes	4	43	47
3. Soft tissue carcinomas			
Total	24	101	125
Lung	2	12	14
Breast	11	11	22
Skin	3	2	5
Others (eg. brain)	2	8	10
Urogenital region			
Total	4	33	37
Bladder	0	8	8
Prostate	0	12	12
Uterus	1	6	7
Fallopian Tube	0	1	1
Ovary	1	2	3
Kidney	2	4	6
Intestinal region			
Total	2	34	36
Stomach	1	13	14
Pancreas	0	5	5
Colon	0	5	5
Rectum	0	5	5
Liver	1	6	7
4. Leukaemia	1	5	6

Table 2. Contact and follow-up of patients in the control group

	Total group number (%)	Females number (%)	Males number (%)
Total	287 (100)	161 (100)	126 (100)
Answered questionnaires	101 (35.2)	54 (33.5)	47 (37.3)
Deceased	24 (8.4)	12 (7.5)	12 (9.5)
Censored	52 (18.1)	33 (20.5)	19 (15.1)
Not answered	110 (38.3)	62 (38.5)	48 (38.1)

Head of project 12: Prof. Dr. A.M. Kellerer

II. Objectives for the reporting period:

There were two major objectives. The first objective concerned the further development of statistical methods and of methods to visualise and intercompare the data on late radiation effects. The second objective was the improvement of the Scheimpflug technique for the quantitative assessment of lens opacifications associated with injections of radium-224.

III. Progress acheived including publications

The wide range and the variability of problems encountered in the statistical analysis of late effects require algorithms that go beyond the standard statistical tools. We have, therefore, generated a set of algorithms within the general framework of interactive data language (IDL). Special emphasis has been on the preparation of diagrams that clarify the dose, age and time dependence of the excess tumour rates. The numerical results are discussed in the report for project 11.

The main effort within the project was on the development of the hardware, software and the measurement techniques for the lens-opacification studies. In order to perform further analysis of the radium-224 induced cataracts among the ankylosing spondylitis and tuberculosis patients, a Scheimpflug camera system has been acquired. This system has, in the past reporting period, been modified by substituting the conventional camera with an electronic camera and an electronic image storing system. The system was then fitted into a mini-van to permit the program of repeated ophthalmological investigations with the Scheimpflug camera without calling the patients to the ophthalmological clinic in Munich. This now permits a more cost-efficient system, involving examinations of patients in their dwellings or in nearby clinics.

In a preliminary phase of the project, before the Scheimpflug system had been modified, an initial survey of patients (largely within the region of Hannover) was performed. This included six weeks of visits to patients and the study of more than thirty patients for lens opacifications. The data acquired in this way were then analysed in terms of the conventional densitometric methods. These data will serve as a basis for future comparisons with the more advanced investigations. The first analysis of these data has been published (1).

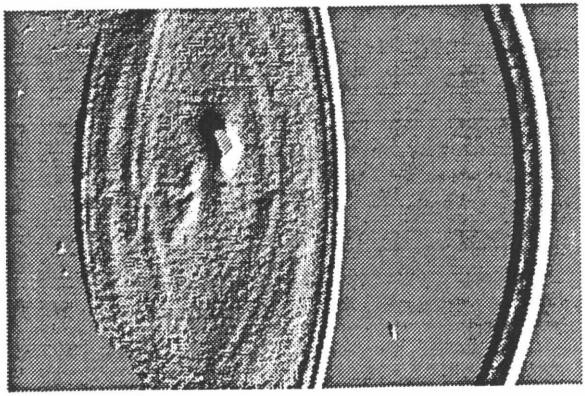


Fig.1: Scheimpflug image of a lens of the eye with cataract near the centre of the lens; the image has been digitally processed.

After modification of the Scheimpflug system, work has been initiated and partly completed on the development of the image analysis system and on the further improvement of the instruments for fast repeated Scheimpflug images at different angles of the same lens. This should ultimately permit a full survey of the spatial distribution of opacifications in the lens and their precise quantification.

A further objective in the past reporting period has been the establishment of a control group for the assessment of lens opacifications and their evolution in dependence on ages. For this purpose 140 males were subjected to Scheimpflug analysis of their lenses within the project. They will be a long-term control group to be analysed repeatedly over the years to come. Altogether 1400 images have now been taken with the new technique within the study. Storage is on optical discs. It is felt that beyond their application to the radium-224 study, the development of these methods will be an aid to the improvement and implementation of the Scheimpflug analysis of lens opacifications in general.

Publications

(1) J.P. Scharff, E. Nekolla, P. Egner, H. Roos, A. Wegener, A.M. Kellerer, H. Spiess, F.H. Stefani. Findings in human lenses 40 years after injection of Ra-224. Accepted for publication in Ophthalmic Research.

(2) P. Egner, H. Roos, A.M. Kellerer. Entwicklung eines elektronischen Systems zur Erfassung von Linsentrübungen mittels der Scheimpflug. Proceedings: 24, Wissenschaftliche Tagung der Deutschen Gesellschaft für Medizinische Physik.

Heads of project 13: Dr. G.M. Kendall
Dr. C.R. Muirhead
Dr. M.P. Little

II. Objectives for the reporting period

To conduct further analyses of data from the first follow-up of the UK's National Registry for Radiation Workers, including assessments of the robustness of the results from the main analysis published previously. To attempt to increase the size of the cohort that will be utilised in the second analysis.

III. Progress achieved including publications

National Registry for Radiation Workers (NRRW)

A further analysis of data from the first follow-up of the NRRW has been published (Little et al, 1993). The main analysis of these data had shown a statistically significant trend in the risk of leukaemia (excluding chronic lymphatic leukaemia (CLL)) with external dose, and weaker evidence of a trend with dose in the risk of all cancers (Kendall et al, Br. Med. J., **304**, 220-225 (1992)). The purpose of the latest analysis was to assess the robustness of the findings in the main analysis to various assumptions on latent period and sub-stratifications, as well as to determine the leukaemia subtypes in which the trend with radiation dose might be most concentrated. Chronic myeloid leukaemia was found to be the leukaemia subtype with the most significant trend with dose (and the largest relative risk coefficient). The sensitivity analyses demonstrated that the significance and magnitude of the trend with dose for leukaemia (excluding CLL) is robust to lag periods of 5 years or less; see Table 1. Both of these findings are consistent with the patterns of leukaemia excess seen in the Japanese bomb survivors and various other irradiated groups and tend to strengthen the likelihood of the observed leukaemia excess being genuinely radiation-induced.

Table 1. Influence of lag period on the test for trend with dose in mortality from leukaemia (excluding CLL) (from Little et al, 1993).

Lag (yrs)	Deaths†	Excess RR Sv^{-1} (+ 90% CI)	Significance‡
Leukaemias excluding chronic lymphatic leukaemia			
0	48	4.33 (0.39,13.28)	p = 0.03
2	47	4.28 (0.40,13.58)	p = 0.03
5	40	2.96 (-0.22,10.19)	p = 0.07
10	35	2.68 (-0.74,11.99)	p = 0.13
15	24	2.89 (-1.19,13.97)	p = 0.17
20	18	3.27 (<-2.06,20.20)	p = 0.22

† Deaths in informative strata
‡ One-sided test for trend based on score statistic

The first analysis of the NRRW was based on a cohort of about 95,000 workers. Work is in progress to extend this cohort, particularly by including groups of past radiation workers whose doses were higher than those received currently. These groups include workers at Nuclear Electric, Scottish Nuclear and Amersham International. As well as increasing the size of the cohort available

for the second analysis, this work should ensure that the collective dose is increased relative to that for the first analysis (namely 3,200 man Sv), as would be the statistical power of the dose-response analyses. Preliminary calculations have suggested that the confidence intervals for the estimates of risk per unit dose in the second analysis should be smaller than those in the first analysis by about 30% in the case of all cancers and 20% in the case of leukaemia (excluding CLL).

Publications

Little, M P, Kendall, G M, Muirhead, C R, MacGibbon, B H, Haylock, R G E, Thomas, J M and Goodill, A A. Further analysis, incorporating assessment of the robustness of risks of cancer mortality in the National Registry for Radiation Workers, J. Radiol. Prot., **13**, 95-108 (1993).

Kendall, G M and Muirhead, C R. An investigation in the United Kingdom of the risks of occupational exposure to radiation. Kerntechnik, **58**, 216-219 (1993).

Kendall, G M, Muirhead, C R and MacGibbon, B H. Leukaemia risks: fact or fiction? Nucl. Eng. Intl., **37**, 42-44 (1992).

Kendall, G M, Muirhead, C R and MacGibbon, B H. The UK's National Registry for Radiation Workers: first results. Nucl. Eng. Intl., Dosimetry and Radiation Protection, 2-3 (1993).

Muirhead, C R, Kendall, G M and MacGibbon, B H. Mortality and occupational radiation exposure: First analysis of the UK National Registry for Radiation Workers. IN Low Dose Irradiation and Biological Defence Mechanisms (eds. T. Sugahara et al), 95-98. Amsterdam, Elsevier Science Publishers BV (1992).

Muirhead, C R, Kendall, G M and MacGibbon, B H. Analysis of mortality based on the UK National Registry for Radiation Workers. Radiat. Res., **133**, 124-126 (1993).

IV. Objectives for the next reporting period

Work on extending the NRRW cohort to include the groups listed above will continue. As the coverage of the desired study population approaches completion, greater attention will be given to commencing the follow-up of the cohort. The latter task will involve extensive contacts with the National Health Service Central Register and the Department of Social Security.

Contract FI3P-CT 930065

Title: Risk estimates of lung cancer from the follow-up of uranium miners

1- Chmelevsky	GSF
2- Tirmarche	CEN-FAR
3- Muirhead	NRPB
4- Darby	ICRF

General Description of the Project

Radon and its daughter products constitute the most important source of natural radiation exposure of the population. There are various animal data on the induction of lung cancer by radon and its daughter products, but neither these data nor epidemiological studies of domestic radon exposure can as yet provide quantitative risk estimates. Such estimates need to be based on studies of uranium miners exposed to higher levels of radon.

Data for major cohorts were recently reviewed in a document of ICRP (ICRP 50) and reevaluated by a special Committee of the US.National Academy of Sciences (BEIR IV). A new evaluation, also by a Committee of the US National Academy of Sciences, is presently under way, it includes, in addition to the most important Western cohorts, the data from the S-cohort of uranium miners which were not earlier available. Results are expected to appear soon.

General aim of our joint project is the improvement of the present risk estimates for lung cancer following radon inhalations.

Objectives for the reporting period.

Consequently our project has a two-fold aim. One objective is the comparison of models to be used in epidemiological studies of lung cancer among uranium miners and the development of algorithms which permit to take into account the relevant variables; i.e. the time pattern of the exposures or the smoking habits and others.

Second aim of the study is the analysis of the available cohorts of uranium miners, the Czech uranium miners (project 1 and 4) the cohort of French uranium miners (project 2) and the miners of the Colorado Plateau (project 3). This includes the examination of confounding factors such as time after exposure or duration of mining. A special effort is being done to

quantify the influence of smoking on the basis of the data from the Colorado Plateau (project 3).

Progress achieved.

Two analyses of the S-cohort of Czech uranium miners have been performed. In both cases they were the result of a collaboration with the Institute for Hygiene and Epidemiology in Prague. The first analysis performed with the group of project 1 was based on a follow-up to 1985. The second analysis, based on an improved and longer follow-up (to 1990), led to conclusions somewhat different from the first analysis.

It appears unclear at this point whether such differences are only due to differences between the data sets or are, at least partly, artefact of models which are not adequate. Important differences in the risk pattern have also been observed between the cohorts analysed by the BEIR IV Committee.

An analysis of the French uranium miners has also been recently completed (project 2). From the results and conclusions of this work one can again note important differences to other cohorts with regard to the risk. This cohort is characterized by long exposures and low annual rates of exposure.

Objectives for the next reporting period.

It appears important for the analysis of data with prolonged exposures to establish models and methods of analysis which have the required flexibility. The analysis of the S-cohort has shown that this is not quite the case for the EPICURE package which was developed for the analysis of survivors of the atomic bombs in Hiroshima and Nagasaki. This aspect of the work is the particular concern of projects 1 and 3.

An effort will be done to extend the cohort of French uranium miners (project 2).

An analysis of the influence of smoking among the miners of the Colorado Plateau is underway. An effort along the same line is planed by the group of project 4 on the basis of the Czech data.

As a further important step the project should be extended to include the analysis of the several hundred thousand miners of the Wismuth AG in Thüringen, Germany.

Head of project 1: Dr. D. Chmelevsky
(GSF Neuherberg)

Title of the project:

Models applicable to the risk of radon exposures and analysis of cohorts of uranium miners

Progress achieved

The analysis of the S-cohort of Czech uranium miners which was mostly performed with the AMFIT program has shown that, apart from cumulated exposures, two other parameters have a very major influence on the risk of lung cancer:i.e. age attained and time since exposure. Alternatively the risk can as well be expressed in terms of age at exposure and time since exposure.

The analysis which was performed with the Poisson regression and with cross tabulated data could not be carried out as far as necessary. The method inherent to a Poisson Regression does not permit to include in a satisfactory way the variable time after exposure when the exposure occurs over an extended time. With a Cox regression which uses the individual information instead of cross tabuled data the computations would be too extensive with the present form of the PEANUT program (which is part of the EPICURE package).

Objectives for the next reporting period

We are now developing an improved algorithm which permits to overcome these difficulties and which should be particularly useful for an analysis where the yearly exposures are considered instead of the total cumulated exposures.

Publication:
A survey of the Czechoslovak follow-up of lung cancer mortality in uranium miners.
J. Sevc, L. Tomasek, E. Kunz, V. Placek, D. Chmelevsky, D. Barclay, A.M. Kellerer
Health Physics 64 (4): 335-369; 1993

CONTRAT CCE FI3-CT93-0065

Proposal IPSN/DPHD/SEGR

Scientific project manager: Margot TIRMARCHE

Project: Contribution of the French uranium miners cohort to the modellisation of lung cancer risk due to exposure to radon and its decay products.

Present state of the study at the end of august 1993:

The mortality of a cohort of 1785 underground uranium miners having begun working underground before1972 and being followed to the 31 december 1985 has been published in the British Journal of Cancer in may 1993 (67,1090-1097). The results of an excess of lung cancer and of laryngeal cancer are discussed in relation to cumulative radon exposure: laryngeal cancer mortality seems not to be linked to radon, but lung cancer mortality is increasing significantly ; the SMR of lung cancer is increased by 0.6 % per WLM if we assume a linear dose-response relationship, the excess relative risk per WLM is close to 0.4 %.

This relatively low excess risk per WLM on a population of miners with a low cumulative exposure (mean = 70 WLM) and low annual exposures does not confirm the hypothesis of an inverse dose-rate effect at low dose-rate.

This French study is also included in a common analysis of 11 cohorts of uranium miners, under the coordination of the National Cancer Institute (NCI) in Bethesda (USA).

An extension of this study is presently organised in collaboration with the medical staff of COGEMA including in a future analysis all the French uranium miners (open pits and underground workers) in order to study on a larger group (about 5000 miners) the dose response relationship between low annual exposures (1-3 WLM per year) and lung cancer risk.

A similar approach will be conducted by LadislaV TOMASEK in Prague, after having discussed the dosimetric survey and vital status survey conditions in these two countries.

Heads of project 3: Dr. C.R. Muirhead
Dr. M.P. Little
(NRPB, UK)

II. Objectives for the reporting period

To conduct preparatory work for analyses of lung cancer mortality among uranium miners on the Colorado Plateau (USA).

III. Progress achieved

Work has commenced to examine a number of issues associated with the risk of lung cancer among miners exposed occupationally to radon. These issues include:

(i) the temporal pattern of risk, which affects estimates of lifetime risk;

(ii) the joint effect of radon and smoking, which affects estimates of risk among smokers and non-smokers separately; and

(iii) the possibility of exposure rate effects, which may affect extrapolations of risk from a mining to an indoor environment.

Examination is also being made of some methodological issues associated with the analysis of data on miners, related to the fact that exposures are received over a prolonged period of time rather than instantaneously.

The above topics are being studied using data on just under 3,350 uranium miners who worked on the Colorado Plateau in the USA. These data, which were reported upon by Hornung and Meinhardt (Health Phys., **52**, 417-430 (1987)), were obtained from the US National Institute for Occupational Safety and Health. Up until the end of 1982, just over 250 of these miners had died of lung cancer. An advantage of these data over those for some other groups of radon-exposed miners is that information on smoking habits is available.

Analyses of these data have commenced, using the EPICURE suite of programs (written by D.L. Preston et al). Initially the AMFIT and PEANUTS programs within EPICURE were used to conduct Poisson and Cox regressions respectively. However, owing to the difficulty in allowing for complex time-varying exposures in the former case and large computational requirements in the latter case, a third program within EPICURE, namely PECAN, is now being used to conduct nested case-control analyses. In order to perform such analyses it is necessary, for each lung cancer case, to sample a set of controls from the miner cohort. This approach has the advantages of:

(i) being able to deal with time-varying exposures, and;

(ii) a large reduction in computational requirements at the expense of a minimal loss of statistical power, by utilising data for a subset of miners rather than the entire cohort.

Contacts have been made with Dr. D.C. Thomas (University of Southern California), who has

used this approach in some analyses of these data.

IV. Objectives for the next reporting period

Detailed analyses of the Colorado Plateau data will be undertaken, using the case-control approach described above. Particular attention will be paid to the joint effect of radon and smoking. In this regard, study will be made of whether the order in which radon and smoking exposures are received influences the lung cancer risk. As well as empirical models, ie. models that attempt solely to describe the pattern of risks observed, it is hoped to apply models based on theories of carcinogenesis to the above data.

Head of project 4: Dr Darby

II Objectives for the reporting period

The objectives for the current reporting period include: working with scientists from the National Institute of Public Health, Prague, to improve both the quality of the follow-up and the quality of the exposure estimates for the cohort of West Bohemian uranium miners, and to commence a series of analyses of the data derived from this study covering risks of lung cancer, other cancers, and other causes of deaths.

The objectives for the next reporting period include further work on the analysis of lung cancer risk and of the risk of mortality from causes of death other than cancer in the cohort of West Bohemian miners and preparation of manuscripts, and an extension of the work on mortality from cancers other than lung cancer to other cohorts of radon-exposed miners.

III Progress achieved including publications

Follow-up of the Population

During the decade up to 1990, follow-up of the West Bohemian miners' cohort mainly relied on the national population registry at the Czechoslovak Ministry of the Interior. However, after the work done to follow the population in this way until 1990, a comparison of the number of deaths observed from causes other than lung cancer or accidents and violence by calendar period suggested that a substantial number of deaths had been missed. Therefore, a series of additional checks were conducted: in the files of the Czech and Slovak Pensions Offices, by local enquiries, and by direct correspondence. These additional efforts resulted in an increase of more than 10% in the numbers men known to have died or emigrated and it has been established that, on the follow-up date of 1 January 1991, 2415 men (55.9%) had died, 314 (7.3%) had emigrated, 1548 (35.8%) were alive and living in the former Czechoslovakia, while only 43 (1.0%) were lost to follow-up. For the men who had died the cause of death was established for all but 41.

Estimation of Exposures

An exceptional feature of the West Bohemian study is the large number of measurements of radon concentrations made in each mine-shaft of the Czechoslovakia uranium mines in almost every year, including the early period. When the cohort was set up, manual calculations were carried out combining these data with the men's employment details to estimate each man's annual exposure to radon progeny in terms of working levels. A recent review of these exposure estimates revealed that there were a number of errors in these manual estimates. In addition some men also worked, particularly after the closure of most of the shafts at Jackymov and Horni Slavkov in 1963 at other Czechoslovak uranium mines and, for some of the men involved, exposures at these mines had not previously been taken into account. Therefore, a major revision of the exposure estimates has been carried out. The revised estimates of final cumulative exposure differed by 5% or less from previous estimates for 35% of the men,

while for 19%, 22%, 20% and 4% of men the differed by 6-10%, 11-20%, 21-50% and over 50% respectively.

Cancers other than lung cancer

The analysis of mortality from cancers other than lung cancer has been completed. It was found that for all cancers other than lung cancer the number of deaths observed was slightly greater than that expected from national rates, but the increase was not significant statistically (ratio of observed to expected deaths [O/E] = 1.11, 95% confidence interval [CI] = 0.98-1.24, see table 1. Mortality did not increase with duration of employment underground or with cumulative exposure to radon. Non-lung cancer mortality was significantly raised among men who started mining work aged under 25 but the increase was not related to cumulative radon exposure. When twenty-eight individual sites and types of cancer were examined, significantly increased risks were found for cancers of the liver (O/E = 1.67) and gallbladder and extrahepatic bileducts (O/E = 2.26). For liver cancer, mortality did not increase with duration of employment underground or with cumulative radon exposure. For cancer of the gallbladder and extrahepatic bileducts, mortality did not increase with duration of employment, but increased with cumulative exposure to radon. Mortality from multiple myeloma, although not significantly increased overall (O/E = 1.08), increased with cumulative exposure to radon. Mortality from leukaemia was not increased overall (O/E = 0.91) and was not increased overall (O/E = 0.91) and was not related to cumulative radon exposure, but did increase with increasing duration of employment in the mines.

There is no strong evidence in these miners that a radon-rich atmosphere increases the risk of any cancer other than lung cancer. Possible exceptions are cancer of the gallbladder and extrahepatic bileducts and multiple myeloma, but further study is needed before it can be concluded that the associations found are causal.

Publications

1. Tomášek, L, Darby, SC, Swerdlow, AJ, Plaček, V, Kunz, E. Radon exposure and cancers other than lung cancer among uranium miners in West Bohemia. *Lancet* 1993; **341**: 919-23.

2. Tomášek, L, Darby, SC, Swerdlow, AJ, Plaček, V, Kunz, E. Epidemiological study of uranium miners in Western Bohemia. To appear in Proceedings of International Workshop on the Health Effects of Inhaled Radionuclides: Implications for Radiation Protection in Mining.

Table 1 Mortality under age 85 in the cohort to 1 January 1991, by site of cancer.

Site of cancer (9th revision ICD code)	Observed deaths	Ratio of observed to expected deaths	95% confidence interval
Lung (162)	704***	5.08	(4.71-5.47)
All cancers other than lung (140-208 excl. 162)	292	1.11	(0.98-1.24)
Tongue and mouth (141,143-145)	3	0.70	(0.14-2.03)
Salivary gland (142)	0	0.00	(0.00-5.43)
Pharynx (146-149)	2	0.51	(0.06-1.83)
Oesophagus (150)	7	1.22	(0.49-2.51)
Stomach (151)	57	1.05	(0.79-1.35)
Intestines excl. rectum (152-153)	18	0.84	(0.50-1.34)
Rectum (154)	24	1.04	(0.67-1.55)
Liver (155)	22*	1.67	(1.04-2.52)
Gallbladder and extra-hepatic bile ducts (156)	12*	2.26	(1.16-3.94)
Pancreas (157)	24	1.40	(0.90-2.08)
Nose (160)	1	1.70	(0.02-9.34)
Larynx (161)	9	1.02	(0.47-1.94)
Bone (170)	2	0.69	(0.08-2.48)
Connective tissue (171)	2	2.11	(0.23-7.56)
Malignant melanoma (172)	6	1.75	(0.64-3.79)
Other skin (173)	2	1.36	(0.15-4.88)
Prostate (185)	15	0.99	(0.55-1.63)
Testis (186)	1	1.10	(0.01-6.05)
Bladder (188)	13	1.15	(0.61-1.97)
Kidney (189)	18	1.30	(0.77-2.06)
Brain and central nervous system (191,192)	9	0.99	(0.45-1.88)
Thyroid (193)	0	0.00	(0.00-3.42)
Non-Hodgkin lymphoma (200,202)	8	1.40	(0.60-2.75)
Hodgkin's disease (201)	3	0.67	(0.13-1.96)
Multiple myeloma (203)	3	1.08	(0.22-3.13)
Leukaemia (204-208)	10	0.91	(0.44-1.67)
Myeloid leukaemia (205,206)[+]	3	1.03	(0.21-3.00)
Other and unspecified	21	1.02	(0.63-1.56)
All cancers (140-208)	996***	2.48	(2.33-2.64)

[+] Data from 1968 onwards only.
* $p<0.05$; *** $p<0.001$ (two-sided)

Progress Report

Contract: FI3P-CT920004 **Sector:** C2A

Title: Development of fundamental data for radiological protection.

1) Smith ICRP

I. Summary of Project Global Objectives and Achievements

The primary aim of radiological protection is to provide an appropriate standard of protection of man without unduly limiting the beneficial practices giving rise to radiation exposure. To achieve this end, it is necessary to quantify the detriment associated with exposure and to develop a general policy of protection of exposed populations and for individuals.

Much of the work of the International Commission on Radiological Protection (ICRP) in the next few years will be a consequence of the new basic recommendations in radiological protection, published in ICRP Publication 60 (1990 Recommendations of the ICRP).

Head of project 1: Dr. Smith

II. Objectives for the reporting period

Task Groups within the four standing committees of the ICRP are providing updated information on radiation-associated risks of cancer and severe hereditary effects; on the application of the basic recommendations in terms of secondary limits; and on the principles of optimisation and management of radiological protection.

III. Progress achieved including publications

Specific programmes of work envisaged or in progress are designed to:-

- characterise the molecular and genetic mechanisms involved in carcinogenesis,
- consider the appropriateness of projection models used in calculating cancer risk and the validity of transfer of estimated risks from one population to another.
- review the evidence on the sensitivity of the embryo and fetus in relation to carcinogenesis
- examine the information available from Soviet epidemiological studies following the Chernobyl accident and on persons exposed to fission products discharged into the environment at Chelyabinsk.

- investigate the possible influence of radiation on the incidence of multifactorial hereditary diseases.

- use the newly recommended human respiratory tract model to provide dose coefficients following inhalation of radionuclides, both by workers and the general public.

- review human anatomical, physiological and biochemical parameters for use in dosimetry and the validity of biokinetic models to describe the metabolism and excretion of incorporated radionuclides.

- revise ICRP Publication 30 (Limits for Intakes of Radionuclides by Workers).

- clarify the conceptual differences between the protection quantities defined in ICRP Publication 60 and field quantities defined by ICRU; and provide fluence to effective dose calculations for a variety of radiations and energies.

- provide a coherent procedure for dealing with protection against radon in dwellings and workplaces, including mines. A draft report has been widely circulated for consultation and the revised report will be discussed by the Commission at its September meeting. Action levels for dwellings and for workplaces are recommended. The proposed annual occupational limit on exposure is 14 mJ h m^{-3} in a year, corresponding to 4 WLM in a year in historical units.

- clarify and extend the previously reported principles for monitoring workers.

- define protocols for protecting the public in a radiological emergency in terms of relocation from contaminated areas and return to decontaminated areas.

- elaborate the concepts of protection against exposures that might occur, including potential releases of disposed radioactive waste over long timescales.

RECENT PUBLICATIONS

An addendum to ICRP Publication 53 (Radiation Dose to Patients from Radiopharmaceuticals). This report provides information on the biokinetics and calculated tissue doses following the intake of six new radiopharmaceuticals now in common use.

A report on the Principles for Intervention for Protection of the Public in a Radiological Emergency updates and extends the advice given in ICRP Publication 40 (Principles for Protection of the Public in the Event of Major Radiation Accidents), particularly in the application of Publication 60 principles to control of contaminated foodstuffs and relocation of population groups.

A report on Protection from Potential Exposure provides a conceptual framework upon which to apply radiological protection principles. There are recommendations for annual probabilities from which constraints on potential exposures might be chosen. This important

document forms an initial bridge between the radiological protection and nuclear safety philosophies, which giving practical guidance that can be used for much simpler sources.

PUBLICATIONS IN PRESS

A report of the Task Group on Age Dependent Dosimetry from the Committee on Secondary Limits will be published as Part 2 of ICRP Publication 56 (Age Dependent Doses to Members of the Public from Intakes of Radionuclides). Doses per unit intake following ingestion will be given for isotopes of sulphur, cobalt, nickel, zinc, molybdenum, technetium, silver, tellurium and polonium using the new tissue weighting factors. New age-specific biokinetic models for the alkaline earth elements and lead, and for plutonium, americium and neptunium will be recommended for use in calculating dose coefficients. Dose coefficients following inhalation of these radionuclides are not included, but they will appear in a future publication, together with updated values for those radionuclides published in Part 1.

A report on a Human Respiratory Tract Model for Radiological Protection will be published. It is based upon increased knowledge of the anatomy and physiology of the respiratory tract and of the deposition, clearance, and biological effects of inhaled radioactive particles.

The new model will:

- provide calculations of doses for members of the public, in addition to workers;
- be useful for predictive and assessment purposes as well as for deriving limits on intakes;
- account for the influence of smoking, air pollutants, and respiratory tract diseases;
- provide for estimates of respiratory tract tissue doses from bioassay data; and
- be equally applicable to radioactive gases as well as to particles.

Addendum to ICRP Publication 53 - Radiation Doses to Patients from Radiopharmaceuticals. Annals of the ICRP 22 (3) 1991.
ICRP Publication 63, Principles for Intervention for Protection of the Public in a Radiological Emergency. Annals of the ICRP 22 (4) 1991.
ICRP Publication 64, Protection from Potential Exposure: A Conceptual Framework. Annals of the ICRP 23 (1) 1993.
ICRP Publication 56, Age-Dependent Doses to Members of the Public from Intake of Radionuclides: Part 2, Annals of the ICRP 23 (2,3) 1993.
ICRP Publication 65, Protection against Radon at Home and at Work. Annals of the ICRP 23 (4) 1993.
ICRP Publication 66, Human Respiratory Tract Model for Radiological Protection. Annals of the ICRP 24 (1–4) 1994.

Progress Report

Contract: F13P-CT920033 Sector: C21

Title: Alara in installations.

1)	Lefaure	CEPN
2)	Zeevaert	CEN/CSK Mol
3)	Pfeffer	GRS
4)	Wrixon	NRPB

I. Summary of Project Global Objectives and Achievements

One of the fundamental principles underlying radiation protection, since ICRP 26, is that all exposures shall be kept As Low As Reasonably Achievable. This principle is an explicit requirement of the CEC Directives laying down the basic safety standards for radiological protection (EURATOM Directive). The new ICRP 60 recommendations have emphasised the role of this principle not only for collective dose but also for the number of people exposed and the distribution of individual doses; it has also introduced the concept of constraints within the optimisation framework.

Four teams (CEN MOL, CEPN, GRS, NRPB) from four CEC members states (Belgium, France, Germany, United Kingdom) are cooperating in this joint project. The area of research covers: - the setting up of Programmes of optimisation of radiological protection in nuclear and industrial installations from the design stage up to the decommissioning of the facility, - further development of tools and software, - as well as transverse themes such as the interface between design and operation, and the interface between radiation protection and safety requirements. This joint project will also take into consideration the impact of ICRP 60 recommendations.

With respect to the development of ALARA tools: - a cost effectiveness, cost-benefit, reasonable-cost software under Windows is now being tested both in English and French versions. Also the main features of an accident and incident data base for the non nuclear sector are now in development using DBase IV and including UK data over the past 22 years; in addition a specific database for radiological optimisation analysis purposes in the framework of dismantling nuclear reactors, including data on dose, costs and waste production, will be derived from existing databases on decommissioning in the EEC.

A CEC Workshop concerning concepts and methods for the integration of social and ethical values in radiological risk management was held in June 1993. This allowed discussions with economists from different European universities, concerning hypotheses and problems concerning the alpha value models (assessment, discounting, irreversibility,...). Work is also ongoing for the development and use of Dose Constraints in occupational, public and medical exposures.

More practical studies involving the nuclear industry are ongoing in France and Germany. They respectively aim at providing a quantification of work management factors to define a model for the assessment of radiation protection options, and at discussing the potential of dose reduction arising from possible changes in test frequencies with respect to safety aspects.

Head of project 1: Dr. Lefaure

II. Objectives for the reporting period

The CEPN objectives for the reporting period were mainly devoted to the development of tools and methodology:

1/ it concerned first a further development of the cost effectiveness/cost benefit software under Windows rather than the less user-friendly DOS environment. As far as this software is concerned the objective was to make it available for beta test in each of the participating organisations by the end of 1993.

2/ secondly CEPN intended to write theoretical papers on aversion, prudence and discounting of the alpha value, taking into account the new framework recommended in ICRP 60.

3/ the third CEPN goal was to pursue an on going study on ALARA and work management in close relationship with the French nuclear industry in order to develop a model assessed upon some practical experiments and surveys.

Objectives for the next reporting period

1/ The cost effectiveness/ cost benefit software will be tested in the different organisations as well as utilised during the next CEC ALARA Course at Ringhals in November 1993. The remarks of the users will be taken into account by the end of 1993 to improve the software and a users guide will then be prepared. This will be followed by a discussion with the CEC concerning modalities of diffusion of such a software among health physicists.

2/ As far as an alpha value is concerned, the objective for the period is first to review the literature and to explore the methodology of the willingness-to-pay approach. Secondly, it is intended to start a feasibility study concerning willingness-to-pay dealing with different situations (medical, public, occupational, short-medium & long term ...) that would identify the key parameters of the model reflecting the utilities corresponding to these different situations.

3/ The results of the previous period will be presented as a new topic during the next CEC ALARA Course. The quantification of the factors will be pursued and their economic counterparts introduced into the model. CEPN will also undertake a study of the organisation and the information systems employed in the management of an outage, from the perspective of the impact on radiation protection.

III. Progress achieved including publications

As expected the cost effectiveness/ cost benefit software has been developed, using Excel under Windows environment. Two versions of the software are available one in English one in French The software includes also the reasonable cost method recently developed by CEPN for implementing any system with several alpha values, as its rationale seems to be more convenient for industrial decision makers, referring to a kind of easy to handle resource to be affected within a budget. The software has been provided for beta test in each of the participating organisations by September 1993.

A CEC workshop, organised by CEPN, has been held in France in June 1993 with the participation of universitary economists from Belgium, France, Germany, Italy, UK, as well as Switzerland and USA. It aimed at discussing some CEPN drafts concerning concepts and methods for the integration of social and ethical values in the radiological risk assessment. The main topic of this work was to discuss a model aiming at providing a reference monetary value system for the man-sievert ensuring an equitable implementation of ALARA. It also demonstrated that, the monetary value of the man-sievert corresponding to the price of goods which cannot be exchanged on a market, the financial market interest rate can't be used as the discount rate for future exposures This workshop has led to the publication in August 1993 of a CEPN draft report and to the submission of articles to reviews

As far as ALARA and work management is concerned, three different categories of factors have been defined: the factors linked to working conditions (ergonomic of work areas, adaptation of tools...), those characterising the operators (qualification, experience level, motivation...) and the factors directly depending on the operations' organisation (tasks planning, general preparation of works...). In order to quantify the impact of these factors, a review of the literature has been performed. It allowed the estimate of the working conditions factors' impacts. The results have been completed by a survey carried out in five French nuclear power plants and focused on three types of operations: primary valves maintenance, decontamination of reactor cavity and some specialised maintenance operations. A first presentation of the results of these study has been done at the REM Seminar at Pittsburgh (12-15 September 1993).

Publications

[1] CROFT J R , LEFAURE C, EGNER K, and SCHNUER K., **Role of optimisation in the management of workers exposure,** 4th European Seminar on Radiation Protection Optimisation, 20-22 April 1993, Luxembourg.

[2] SCHIEBER C., SCHNEIDER T., **Concepts and Methods for the Integration of Social and Ethical Values in Radiological Risk Management,** CEPN Report n° 217 (draft), 1993.

[3] SCHIEBER C., **ALARA and Work Management,** In: Radiation Exposure Management (REM) Seminar, Pittsburgh, USA, 12-15 September 1993.

[4] LEFAURE C, LOCHARD J., SCHIEBER C., SCHNEIDER T., **Reference Monetary Value System for the Man Sievert: Equity and Time Dimensions,** article proposed to Health Physics, 1993.

[5] SCHNEIDER T., SCHIEBER C., EECKHOUDT L., **Equity and Radiological Risk Management**, article proposed to Risk Analysis, 1993.

Head of Project 2 : Dr. Zeevaert

II. Objectives for the reporting period

The programme of work the SCK•CEN intends to carry out in the project "ALARA in Installations" is directed towards the radiological optimisation of dismantling/decommissioning.
A serious problem in this domain is the lack of data for various important radiological protection factors. The appropriate means to overcome this problem in the future is the establishment of a data base in which relevant data from dismantling operations executed, are collected.
In a first period the objective of the SCK•CEN work has been to identify the important radiological protection factors and the domain in which data bases would be most useful. Already existing international data bases concerning decommissioning/dismantling were identified and their relevancy and usefulness for radiological optimisation purposes investigated.
The primary objective for the second 6 month period will be to investigate how the existing data base selected can be transformed in a data base adapted to the requirements for carrying out a radiological optimisation analysis.
In the framework of ALARA decision-aiding, a multiple attribute type of analyses is to be considered as a general applicable quantitative decision-aiding technique. Decision-aiding tools based on such type of analyses will be examined and an example of the application on specific dismantling operations will be shown.

III. Progress achieved including publications

Among the major steps in a radiological optimisation procedure, the quantification of the relevant radiological protection factors and their application in a quantitative decision-aiding tool posit some special problems in the case of dismantling operations of nuclear power reactors (PWR). Difficulties in the quantification of radiological protection factors are due to a lack of experience in dismantling operations. Collection of data already available in this domain is an obvious aid in order to facilitate such quantifications in the future. The requirements for such a data base with a view on the application of the data in decision-aiding techniques is being studied at SCK•CEN.

Major radiological protection factors that are to be considered with relation to the decommissioning or dismantling of nuclear PWR reactors are :

- Collective Dose
- Distribution of Individual Doses
- Economical Costs :
    - Labour Costs
    - Investment Costs
    - Consumable Costs
- Waste Production :
    - Quantities (Masses - Volumes)
    - Concentrations (Radioactivity)
    - Physical Forms

which may be transformed into

Waste Costs (for treatment, transport, disposal) and
Waste Doses (to operators and population).

An illustration of how these factors may fit in a hierarchy of attributes to be considered in a multi-attribute type of analysis is shown in Fig. 1.

Factors for which quantitative information from a data bank are most useful are :
- duration of work for dismantling tasks, applying specific techniques ;
- costs of equipment ;
- quantities and physical forms of wastes produced (in particular : secondary waste).

Since the values of these factors do not only depend on the operation or task considered but also on the conditions under which the operation has to be executed, the data have to be fully specified in terms of conditions they are related to. If possible normalised values (with relation to reference units) should be specified in order to facilitate derivation of values for other conditions. Deterministic values for these factors are not primordial, uncertainty ranges (pdf when available) are more useful with a view on the testing of the robustness of the solution.

An inquiry has been made concerning existing international data bases on decommissioning/dismantling in Europe. Two such programmes were identified ; the OECD/NEA co-operative programme on decommissioning and the data bases of Working Group C (decommissioning projects) of DGXII of the CEC.

The first programme was established in 1985 with a view to exchange information and experience between decommissioning projects. Detailed studies are being performed in three technical Working Groups :
WG1 : on decommissioning costs
WG2 : on recycling and re-use of dismantled material
WG3 : on decontamination prior to dismantling.
Each working group has established or is establishing its own data base. Their contents is confidential and non-members of the groups normally do not have access to them.
The type of information available in these data bases concerns large management items (WG1) or procedures for decontamination (WG3) and is not suited to the optimisation of specific dismantling operations. So they will not be considered further.

The second programme identified seems to be more promising for our intentions. In the programme of Working Group C of DGXII (CEC) two data bases have been established, namely
- DB-COST : on costs and radiation exposure
- DB-TOOLS : on equipment for dismantling.

They have been organized in ORACLE software and are intended to be coupled to each other later on.
The most interesting data base for our purposes at this stage is DB-COST, drawn up by NIS-Hanau since 1990. It is a very comprehensive data base with

much more information about decommissioning projects than what is really needed for radiological optimisation purposes. On the other hand very valuable information, including specific values on costs, wastes ... related to reference units, is also present. However, data were difficult to acquire and a lot of compartments do not yet contain data.
Our proposal is to collaborate further with Group C and NIS in order to investigate the feasibility of deriving from DB-COST, a data base for radiological optimisation analysis purposes, organized in the more user-friendly ACCESS software.

Publication

[1] P. GOVAERTS, Th. ZEEVAERT, **Integration of the ALARA principle in decommissioning activities**, presented at: 4th European Seminar on Radiation Protection Optimisation; 20-22 April 1993, Luxembourg.

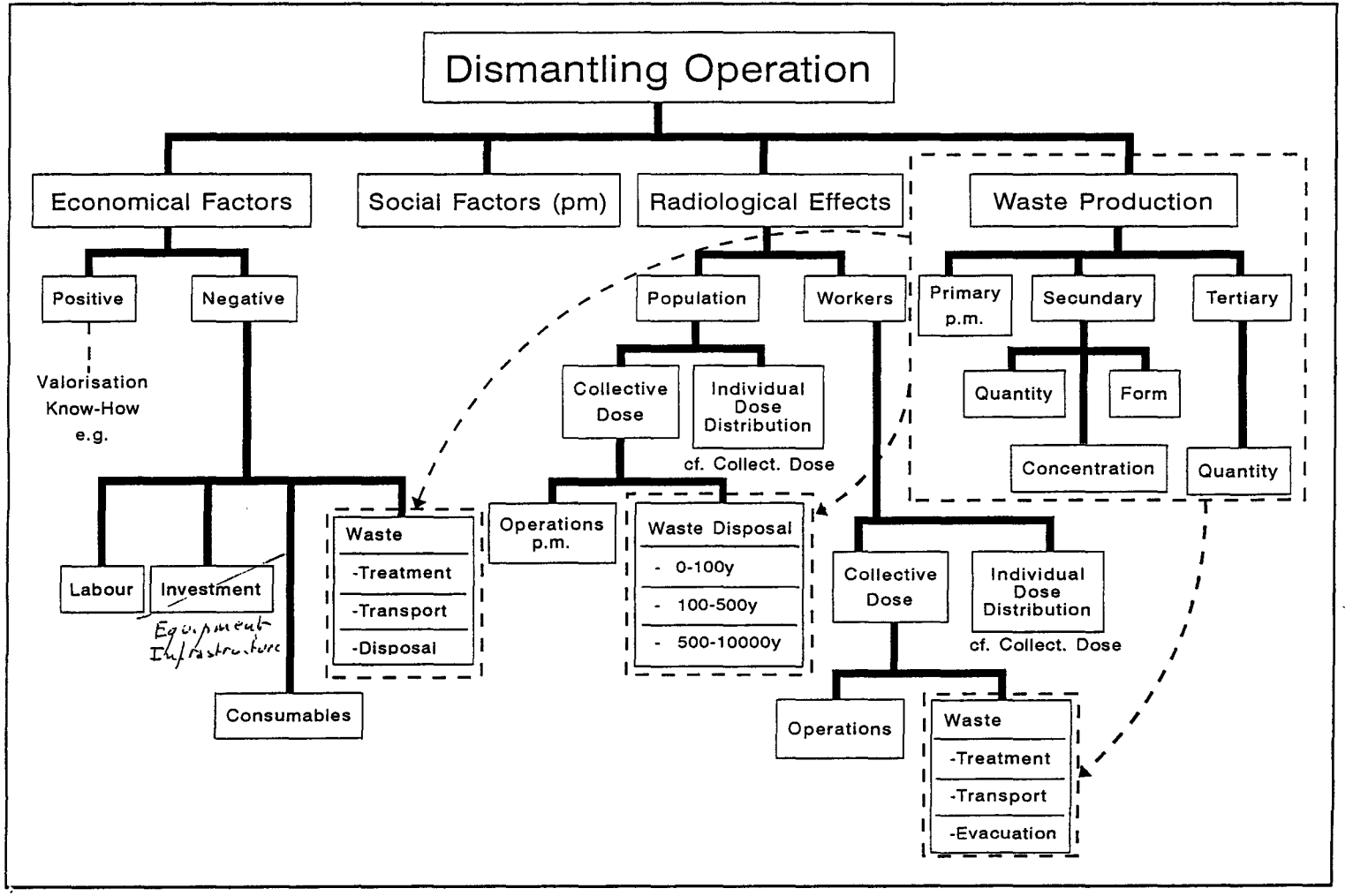


Fig 1. Hierarchy of attributes - Example

Head of project 3: Dr. Pfeffer

II. Objectives for the reporting period

The general objective of the project is to evaluate the dose contributions of activities in testing, inspection, service and repair of NPP to derive recommendations to optimise these activities with respect to the necessity, the frequency and the timing and co-ordination of the tasks.

The main objective of this reporting period was to prepare the data base for the further evaluation. In practice this means especially to collect the data from the NPP under consideration. In co-operation with specialists in the plant activities causing important contributions to the collective dose of the personnel were to be identified considering also frequencies of the tasks. Documents to identify important parameters contributing had to be collected for further evaluation. Additionally the breakdown of tasks in inspection and testing had to be discussed with the personnel regarding activities to prepare the workplace, to install supporting equipment or tools, to perform the inspection and to reactivate the system under consideration. To complete the picture the doses of tasks carried out in several years were considered to be applicable as a basis to evaluate the effect of improvements carried out in the plant.

Objectives for the next reporting period

The next step of the project will be to evaluate the data collected according to the aims of the project. In this evaluation a close co-operation with the plant will be necessary too as we feel the need to refine the information to complete the data base for special aspects under consideration.

The evaluation of the data will consider several areas:

- the dose contributions for special activities carried out in the plant shall be derived as a basis for further consideration and comparison,
- the effect of improvements carried out in the plant up to now shall be pointed out to judge further recommendation and the transfer of experience,
- taking the dose contributions in special activities as a basis, the potential of dose reductions shall be discussed considering changes in testing frequencies, alignment of testing cycles and optimising the co-ordination of activities. These parameters certainly will have to be judged considering safety aspects of the plant influenced by testing and inspection.

The results of the evaluation will be summarised in recommendations to optimise the radiation exposure of the personnel.

III. Progress achieved including publications

Due to the contract starting for GRS in 1993 in the reporting period it was only possible to prepare the data acquisition, to carry out a first data acquisition campaign in the plant, and to perform some very preliminary evaluations. So this summary mainly will present a general overview and information on the data acquired up to now and will give a sketch of the

results expected from the evaluation of the data As mentioned above, during the evaluation the database will be refined in co-operation with the plant to the extent necessary.

In co-operation with the specialists of the plant under consideration the documentation of the plant was reviewed to identify regions of interest considering the aim of the study and to judge the best way of transferring the information available in the plant.

Due to the time scale of the visit of the plant the data collection in this first visit focused on four main fields:

- Regarding the main activities causing contributions to the collective exposure of the personnel in the plant we decided to focus on some important tasks performed at the steam generator, as it is not possible to judge all activities performed. For this tasks documents are available for several years giving the dose rates at places in the vicinity of the steam generator, the time used for the personnel for the tasks performed and the doses contributing. Further information necessary will be discussed with the plant personnel in the evaluation.
  The data will be evaluated to derive the dose contributions from several activities at the steam generator to judge the potential for dose reduction considering different aspects as testing frequencies or the harmonisation of the cycle of inspections.
- The relevant data - to be understood as jobs performed, personnel and time necessary and doses applied - of all activities carried out at the TH- and the TA- systems of the plant under consideration have been collected for the last 5 years. These data have been implemented as a data set on a personal computer. The data set can be processed with a spread sheet program to be able to evaluate these data. In this way it is possible to look for tasks and doses considering the systems, special components, and redundancies. It will be possible to check tasks carried out at the same component in one year, or to compare tasks carried out at the same or redundant component in several years to a certain extent the data should also give an impression of those tasks relevant for the preparation of tests or inspection.
  The data will be evaluated with respect to the doses applied in special activities as a bases for further consideration in the judgement of doses possibly saved due to modification of testing frequencies or due to the alignment of cycles or co-ordination of activities. As necessary further information regarding tasks to be evaluated in more detail will be available from the plant on request.
- The documentation of the plant giving the testing frequencies for all systems and components was taken from the plant and transferred to a file which can be evaluated by a spread sheet program. The file not only contains the testing frequencies of systems and components due to different demands as mechanical or electrical tests, but also the location in the plant defined by room number and location in the room.
  This data set will allow to identify the different testing cycles relevant for systems or components due to different demands as mechanical tests of the component, electrical tests of the drives, special inspections and so on, which are not necessarily harmonised. The kind of test or inspection prescribed is given too. Additionally the data will allow to identify testing activities to be performed at components in the same room or even in the same room area which might give rise to harmonisation, if safety aspects would not be interfered with.
- For the last years the accumulated collective doses for activities in the plant broken down to each of the systems of the plant are available. These data also give the numbers of persons (utility, contracted) involved, the collective doses of the personnel groups, and the dose rate (mean and maximum) at the workplace. These collective doses certainly only will present an overview, as information on components are not

contained, but because of the time dependence of the data it is expected to give some hints regarding the influence of improvements carried out. For this reason the data will have to be evaluated applying the data base already available at GRS containing all activities in the plant giving important contributions to the collective dose. The time scale of plant improvements - as e.g. replacement of Co and Sb or design modifications - are known from the yearly reports of the plant, so the data may help to derive the effect of improvements in the plant in the last years, which might support the decision of plants when judging the benefits of improvements in the optimisation process.
In the evaluation of these effects further data and information may be necessary to be discussed with the specialists of the plant to expand the knowledge.

As stated above only a very preliminary evaluation of the data could be carried out.

Looking at the documentation of the test cycles of components it is quite obvious that testing cycles due to mechanical and electrical demands are different. For instance testing cycles at the same component may be 4, 6 and 8 years for the electrical and mechanical part depending on the test or inspection to be performed. Additionally the testing cycles of different components of the same system located in the same room differ. Additionally to the 4, 6 and 8 year duty cycle discussed, a 5 year cycle holds for safety relief valves. An additional aspect may be the test cycles for the components of different systems located in the same room or same area. Certainly the work management of the plant already aims at harmonisation of these parameters, but from this point of view it will be an interesting aspect to check whether there might be further potential of dose reduction due to harmonisation of testing cycles as well as due to co-ordination of tasks at the same place and to quantify the reduction. Certainly the changes of the test frequency will have to be judged with respect to the effect on failure probability in this case too.

Due to the early stage of the work in the project, there are no publications up to now.

Head of project 4: Dr. Wrixon

II. Objectives for the reporting period

1 To further develop the integration of 'ALARA tools' into a Work Management approach to optimisation.

2 To develop methodologies for the derivation of dose constraints and how they can be practically implemented into radiation protection programmes.

3 To develop an accident and incident database for the non-nuclear sector.

III. Progress achieved including publications

Work Management Approach to Optimisation

NRPB and CEPN provided the lead authors of the invited Issues Paper[1] that set the scene for the session on Occupational Exposure at the CEC 4th Seminar on Radiation Protection Optimisation in Luxembourg, April 1993. This provided an opportunity to bring together many of the threads of the work of the project and to produce a coherent review of the role of optimisation in the management of work exposure. Whilst the paper reiterated the value of the structured approaches to optimisation developed in previous CEC Contracts the principle focus was the development of a work management ethos, covering management commitment, appropriate organisational structure and the motivation, awareness and training of staff. The paper also identified questions that still need to be addressed, many of which relate to the ongoing work of the project.

An additional NRPB paper[2] at the Seminar addressed aspects of optimisation in the non-nuclear sector, eg, industrial medical and research work. It looked at some dose distributions in Europe, the potential for further optimisation and some of the approaches used in the UK to realise this potential.

The practical application of optimisation in work involving potential internal exposure is one area that needs further consideration. Work has started on reviewing the approach taken in practice in this area by a variety of organisations in different work sectors. The intention is to develop guidance that would allow an optimal balance where there is a trade off between a variety of factors, for example well characterised external irradiation and uncertain internal exposure. Work has been published[4] giving a new view of the categorisation and designation of working areas in which unsealed radioactive material are used. How this might be used as an aid to optimisation is being reviewed.

Development and Use of Dose Constraints

Work has been carried out on the function, derivation and use of dose constraints in occupational[5], public[6] and medical exposure[7]. This work identified that the dose constraint is seen as having slightly different functions in each of these areas. In particular for occupational exposure it was clarified that the dose constraint acts as an upper bound on the optimisation process and should be used in a prospective sense. However it was recognised that during operations there may be situations where the real doses exceed these values and that here the numerical values may be used as investigation levels.

In parallel to this, work has been started on the development of advice on the choice of dose constraints in the non-nuclear sector. Here the NRPB is analysing dose data, for the 800 organisations for which it acts as Radiation Protection Adviser, to produce dose profiles for general areas of use. The objectives are to identify driving forces behind the upper end of dose distributions and to review factors which managements should taken into account in choosing dose constraints.

Accident and Incident Database

Learning from our past experiences (the feedback loop) is an important element in any approach to optimisation, and is particularly so in the case of accidents and incidents. Appropriate databases will facilitate this feedback and provide the input to the development of methodologies to derive risk constraints. The NRPB is developing an appropriate database using dBase IV software. The first phase uses historical data from the accidents and incidents in the UK that NRPB has had involvement in over the past 22 years.

A list of proposed fields and an associated data element dictionary have been developed. The fields are designed to categorise the nature and cause of the accident in a way that will allow statistical analysis, and provide a text description of the accident in a sanitised form. The latter will also form the basis of a publication providing feedback to users of radiation. In the longer term it is envisaged that other organisations will contribute to and be able to interrogate the database.

Publications

1 Croft J R, Lefaure C, Egner K and Schnuer K. Role of Optimisation in the Management of Worker Exposure. 4th European Seminar on Radiation Protection Optimisation, 20-22 April 1993, Luxembourg.

2 Croft J R. Optimisation of Radiation Protection in the Non-Nuclear Sector. Ibid.

3 Robb J D and Webb G A M. Values of Unit Collective Dose for use in the 1990s. Documents of the NRPB, Vol 4, No. 2 1993 (pages 75-80), Chilton, UK.

4 Hudson A P and Shaw J. Categorisation and Designation of Working Areas in which Unsealed Radioactive Material are used. NRPB-M443, Chilton, UK, 1993.

5 Wrixon A D, Croft J R, Hudson A P and Robb J D. Occupational Exposure: Guidance on the 1990 Recommendations of ICRP. Documents of the NRPB, Vol 4, No. 2 1993 (pages 1 to 26), Chilton, UK.

6 Morrey M E, Robinson C A, Simmond J R, Haywood S M and Cooper J R. Public Exposure: Guidance on the 1990 Recommendations of ICRP. Ibid (pages 27 to 41).

7 Shrimpton P C, Wall B F, Croft J R and Webb G A M. Medical Exposure: Guidance on the 1990 Recommendations of ICRP. Ibid (pages 43 to 74).

Progress Report

Contract: FI3P-CT920014 Sector: C22

Title: Digital Medical Imaging: Optimization of the dose for the examination.

1) Malone Hosp. Federated Dublin Voluntaries
2) Faulkner Hosp. Newcastle
3) Busch Univ. Heidelberg

I. Summary of Project Global Objectives and Achievements

The project this year made significant progress with respect to it's objectives in the areas of image quality and patient dose. Specifically, a variety of coordinated approaches to the image quality problem are well underway including clinical assessment of images from different digital radiographic (DR) modalities, semi subjective contrast detail studies, objective assessment of qualities such as resolution and noise and finally development of the software for more robust objective indices of image quality such as the Wiener spectrum. Based on these studies some conclusions with respect to the suitability of various techniques of DR for specific clinical examinations of the chest, abdomen and pelvis have been reached.

Studies of image quality have been paralleled by dosimetric studies, again at a broad range of levels across the coordination group. These include studies of dose to the chest, abdomen and pelvis in DR, studies of the implications of ICRP-60 and organs included in the field of view in investigations of the GI tract, and studies of organ doses for fluoroscopy of the abdomen. Arising from these it is possible to suggest some tentative reference dose values for DR. In addition staff dosimetry studies have been undertaken with particular reference to the relationships between effective dose and the personnel monitor reading.

Additional work is in progress in several areas within the ambit of the project objectives including, for example, optimization strategies for DSA, constancy checks and write off criteria for digital fluoroscopy systems, studies of hard copy devices and software based/automatic exposure selection systems. Finally, the participants continued coordination activity within the project at a satisfactory level.

Head of project 1: Prof. Malone

Objectives This Reporting Period

* To develop software tools to measure Wiener Spectra, and to measure such spectra from different imaging modalities.

* To assess the contribution to image non-uniformity and distortion directly attributable to multiformat imaging and viewing conditions.

* To continue the presently existing study into the mechanism and design of AEC.

* To initiate a dosimetry study into patient organ doses during various GI examinations, using diamentor readings.

Objectives Next Reporting Period

* To establish and model the relationship between image noise and image intensifier entrance exposure for various II-TV systems using clinical exposure factors.

* To incorporate SNR and MTF measurements into this model.

* To compare organ doses for operator techniques during GI examinations and to present a view of the extent to which the organs, newly identified by ICRP60, feature in these studies.

* To initiate a study into staff dosimetry during cardiac procedures using digital cardiac imaging, screening and cine methods.

* To initiate image quality work on film based imaging techniques using a high resolution film digitizer.

Progress Achieved

Image Quality Studies:

Image quality assessment techniques are currently being evaluated. The use of phantoms such as resolution test patterns (eg. Huttner Phantom) are well established and are widely applied in routine quality assurance programmes. However, in the context of optimization studies, it would appear that such devices are limited by the finest resolution on the test device and hence may lead to incorrect optimization conclusions. Thus more robust techniques (eg. Wiener Spectra, Modulation Transfer Function etc.) suitable for use in optimization studies are being employed.

Wiener Spectra/Signal to Noise Ratios (SNR):

Software tools have been developed to measure Wiener Spectra in both digital and conventional imaging systems. A comparative survey has been initiated between various imaging modalities such as conventional fluoroscopy, digital vascular imaging, digital cardiac imaging, computed tomography and nuclear medicine. These Wiener Spectra measurements are being used to model the image noise / dose relationship, thus providing an objective open ended endpoint to assess image quality.

The increased use of dedicated digital imaging systems is seeing the replacement of film-based imaging techniques. This is particularly evident with cardiac imaging. In some cases this is being implemented with direct replacement of the cine suite with totally digital systems or with the use of add-on computers to existing systems. Assessment of such systems indicate possibilities for dose reduction. However, with regard to image quality, although there is a reduction in limiting resolution there can be considerable improvement in the low-contrast sensitivity, indicating the success of noise reduction techniques and other processing which can be employed.

Image Digitization / Workstations:

An image quality programme is being initiated to assess film based imaging. This requires the use of a high resolution film digitizer and various image processing facilities. Measurements of SNR, Wiener Spectra and other image quality indices are being performed. This should yield a comparison between the film based and digital imaging modalities and it is hoped will give insights into dose limits and optimization.

Multiformat Imaging:

For most digital high technology imaging methods the final copy is generally produced on a multiformat camera. A protocol is currently being developed for performing quality assurance on these devices. The protocol allows assessment of the hard copy image and the camera display monitor fluctuations. Indications are that there is a large degree of variation in image quality with these cameras. Clearly there is a need for standardised routine quality assurance measures. However, there is very poor accessibility to components of the system to enable proper quality assurance measures to be conducted.

Automatic Exposure Control (AEC):

In fluoroscopy and digital imaging modalities use of automated exposure control facilities are almost universal; both for dynamic and hard-copy images. Studies into the mechanisms of AEC and the impact on image quality using SNR measurements have been ongoing for the different modalities. Results indicate variations in the measured SNR with respect to the receptor entrance exposure. However the variations were not reflected when subjective assessments, such as limiting resolution test phantoms, low contrast detectability etc., were used. Exposures measured at the image receptors were seen to vary greatly for the different modalities. Considerable differences appear to exist from system to system depending on the manufacturer and a survey has been conducted with the operators to establish ease of system operation and knowledge of the rationale for AEC. Results indicate that with the number of different manufacturer's systems operating in a department considerable confusion exists and clearly demonstrates a need for standardisation in the design

of such systems.

Viewing Conditions:

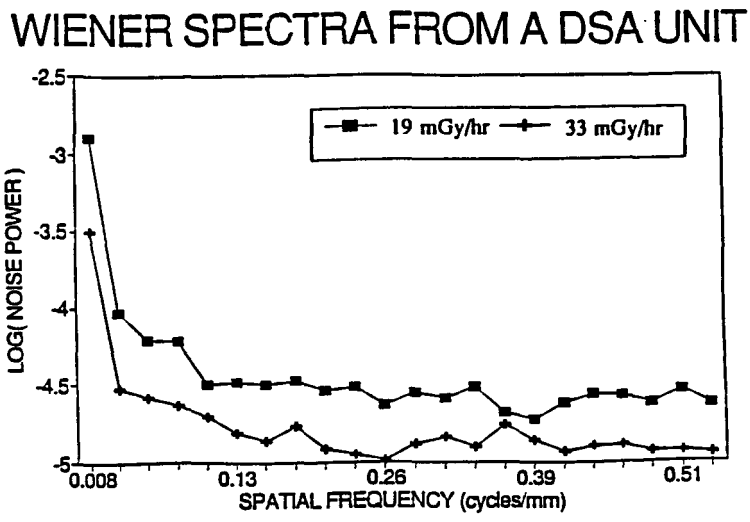
Due to the fact that the majority of diagnostic radiographs, digitally produced or other wise, are viewed as a hard copy image, it is important that the viewing conditions for such hard copies are optimised. On the basis of the EC working document a survey was carried out on the standard of a number of viewing boxes in diagnostic use. It was found that none of the viewing boxes studied fulfilled all of the EC criteria and over 70% of them did not meet any of the criteria. The survey has initiated investigations into simple methods of improvement to the viewing boxes. In addition a protocol is currently being established for simple routine quality assessment of the viewing boxes.

GI Fluoroscopic Times:

Patient organ dose in fluoroscopy is difficult to measure in the upper and lower GI tract. A study is in progress to measure the screening times for organs exposed during the course of an examination and to estimate the energy imparted using diamentor readings. It is ultimately hoped to compare these organ doses for different operator techniques and to present a view of the extent to which the organs, newly identified as important by ICRP 60, feature in these GI studies.

Dosimetry:

Some collaborative dosimetry work has been performed on digital and conventional, chest and abdomen radiographic techniques, using anthropomorphic phantoms and thermoluminescent dosimeters.



Publications:

1. **Radiation Protection Problems with Dental Radiological Equipment.**
   Cooney P., Rajan J., Gavin G. and Malone J.F.
   (Accepted for presentation at 'Data Analysis in Quality Control and Radiation Protection of the Patient in Diagnostic Radiology and Nuclear Medicine', Commission of the European Communities' Workshop, Grado, Italy, September 1993).

2. **Survey of Image Viewing Conditions for X-Ray Film.**
   Cooney P., Davis C., van der Putten W. and Malone J.F. (op. cit.).

3. **Automatic Exposure Control in Fluoroscopic Imaging.**
   Cooney P. and Malone J.F. (op. cit.).

4. **Variations in Image Quality and the Impact of Quality Assurance in Multiformat Cameras.** Marsh D.M., Cooney P. and Malone J.F. (op. cit.).

5. **Measurement of Wiener Spectra in Digital Systems.**
   Marsh D.M., Cooney P., McMahon B.P. and Malone J.F. (op.cit.).

6. **Quality Assurance Programme Applied to Mobile X-Ray Equipment.**
   Tuohy B., Cooney P., Tuohy G., Moran B. and Malone J.F. (op. cit.).

7. **A Practical Approach to Implementation of a QA Programme for Radiology at the Constancy Check Level.**
   Moran B., Upton J., Cooney P. and Malone J.F. (op. cit.).

8. **Optimisation of Radiographic AEC System Performance.**
   Upton J., Moran B., Marsh D.M., Malone J.F. and Cooney P. (op. cit.).

9. **Writing-Off Fluoroscopy Equipment - Impact of Patient Dose and Image Quality in Practice.** Malone J.F., Busch H.P., Faulkner K. (op. cit.).

10. **Image Quality Criteria Applied to Digital Radiography.**
    Busch H.P., Faulkner K, Malone J.F. (op. cit.).

11. **Assessment of Organs in the Field of View during Fluoroscopic Studies of the G.I. Tract.** Quinn A, Wallis F, Molloy M, Upton J, Malone J.F. (op. cit.).

12. **Daylight Film/Processor/Loader Systems - Aspects of Performance and Distribution.** Upton J, Moran B, Duggan M, Malone J.F. (op. cit.).

13. **An Investigation of High Patient Dose for the Lumber-Sacral Projection using AEC.** Moran B, Upton J, Malone J.F. (op. cit.).

Head of project 2: Dr. Faulkner

Objectives This Reporting Period

* Establish reference dose values for digital imaging of the chest, abdomen and pelvis. Compare values with conventional techniques.

* Determine the relationship between effective dose to personnel and monitor reading for fluoroscopy.

* Measure the contrast detail performance as a function of dose at the image receptor for a photostimulable storage phosphor and image intensifier based digital radiology system as well as in digital fluorography and digital subtraction angiography.

* Measure normalised organ dose data for the circular field sizes used during fluoroscopy studies of the abdomen.

Objectives Next Reporting Period

* Develop a method of assessing patient entrance dose-rate and air-kerma rate at the image receptor input surface for fluoroscopy and digital imaging systems.

* Initiate an intercomparison on fluoroscopy and digital imaging systems to establish norms for the above measurements.

* Commence a large scale patient dose survey in fluoroscopy.

* Undertake a regional survey of personal protective devices provided in x-ray departments.

* Optimise staff protection in fluoroscopy and digital imaging.

Progress Achieved

With the advent of digital systems there now exists a range of imaging techniques which may be used to acquire images of the chest and abdomen. An investigation has been performed to determine typical radiation doses associated with the use of different imaging techniques for chest and abdominal radiology. For chest radiology the use of conventional film/screen, 100mm camera, large field image intensifier digital radiography and a scanning slit system were compared. Where as in abdominal radiography, various field sizes used in an image intensifier television system based digital radiography unit were compared with a conventional film/screen, 100mm camera and a phosphor storage computed radiography system.

For chest radiology, radiation doses to radiosensitive organs were assessed using direct measurement made with lithium thermoluminescent dosemeters, together with calculations made using normalised organ dose data. Doses were assessed using anthropomorphic phantoms for both postero-anterior and lateral projections. A similar approach to patient

dosimetry was adopted for the investigation of doses from abdomen radiology. The main difference being the use of normalised organ dose data measured for circular fields commonly used during fluoroscopy.

Based on a comparison of effective dose, it was discovered that both the scanning slit and 100mm camera could reduce doses by a factor of 6 for a PA chest examination. Image intensifier based digital radiography systems could reduce the effective dose equivalent by a factor of 5 compared to a conventional film screen system.

In fluoroscopy, it is usual for individuals to wear protective clothing made from lead rubber. The purpose of these aprons is to shield radiosensitive organs in the trunk, unfortunately, some organs in the head and neck remain unshielded. Individuals are usually issued one personal dosemeter to monitor effective dose. However, there is a divergence of opinion as to the most appropriate monitoring site. The change in dose quantities proposed by the International Commission on Radiological Protection (ICRP) has important implications for personnel monitoring during fluoroscopy. It was decided to experimentally determine the relationship between effective dose and monitor reading for simulations of fluoroscopy. Both overcouch tube/undercouch image intensifier and undercouch tube/overcouch image intensifier geometry equipment were considered. It was deduced from the results of this investigation, that a single dosimeter worn under a lead apron would provide a better estimate of effective dose than a dosemeter worn above the apron. It is possible to combine to dosemeter readings (one worn above the apron, the other below) to provide an improved estimate of effective dose.

Further experimental studies have been performed to experimentally assess the relationship between equivalent dose to the uterus and personal monitor reading for simulations of fluoroscopy procedures. It was discovered that a dosemeter worn at waist level may be used to determine whether the supplementary dose limit for the equivalent dose to the surface of the abdomen of a pregnant member of staff has been exceeded.

The group has also had an input into the International Electrotechnical Commission's Group drawing up standards for acceptance tests on general x-ray equipment mammography units and digital subtraction angiography (IEC 1223 parts 3.1, 3.2, 3.3). A fourth standard for (DSA) digital imaging systems is to be considered.

Publications:

1. **Quality criteria, tolerances, limiting values, dosimetry and optimisation in a number of Fluoroscopic, DSA and Digital Radiological Systems in 'Digital Radiography' Eds. Busch HP and Georgi H. 1992 (Schentztor-Verlag Koustanz.**
   Faulkner K, Marshall NW, Kotre CJ, Chapple CL, Harrison RM.

2. **Personal monitoring during fluoroscopy. Journal of Radiological Protection. 1992 12 (4) 225-231.**
   Faulkner K.

3. **Analysis of variations in contrast detail measurements performed on image intensifier-television systems. Physics in Medicine and Biology. 1992 37 (12) 2297-2302.**
   Marshall NW, Faulkner K, Kotre CJ, Robson KJ.

4. **The relationship of effective dose to personnel and monitor reading for simulated irradiation conditions. Health Physics, 1993 64 (5) 502-508.**
   Faulkner K, Marshall NW.

5. **A quantitative test object for optimization studies in image intensifier television digital imaging systems. Radiation Protection Dosimetry (in press).**
   Marshall NW, Faulkner K, Busch HP.

6. **The implications of ICRP60 on the monitoring of pregnant hospital staff. Commission of the European Communities Radiation Protection Programme Meeting 12th-14th May 1993, Villegin, Switzerland. Abstract in Conference Abstract Book.**
   Chapple CL, Faulkner K, Marshall NW, Rawlings DJ.

7. **Contrast detail performance of various x-ray digital imaging systems. Institute of Physical Sciences in Medicine Meeting. The assessment of x-ray digital imaging systems. 29th June 1993, Birmingham. Abstract in Conference Abstract Book.**
   Marshall NW, Faulkner K, Busch HP, Lehman DJ.

Head of project 3: Dr. Busch

Objectives This Reporting Period

One of the main objectives for the reporting period was the selection of suitable clinical factors for digital intensifier radiography and digital storage phosphor radiography. One aim of our study was to lower the dose as far as possible. This optimisation was performed for the gastrointestinal tract and the lung. Strategies were developed to optimise the dose for DSA. Patient and staff doses were measured to compare analog and digital imaging methods. The clinical value of five analog and digital methods were assessed using both clinical and phantom studies. The parameters for constancy tests for digital image intensifier radiography was studied and a protocol for constancy tests was elaborated.

Objectives Next Reporting Period

Further clinical applications of digital imaging have to be studied. Recommendations for optimization of dose for DSA has to be developed. Dose optimization criteria for digital image intensifier radiography have to be extended to storage phosphor radiography. Further dosimetry of staff and patients is necessary. Quality Assurance Criteria have to be applied to storage phosphor radiography.

Progress Achieved

By comparative studies we have shown that digital image intensifier radiography is a superior imaging method for nearly all fluoroscopic examinations. It is only inadequate when large area imaging with high spatial resolution (eg, lung) is required. Imaging tasks can be placed in one of three categories of image quality: high, medium and low tasks requiring high image quality are examinations of the gastrointestinal tract in double contrast technique. Tasks requiring medium image quality are Cholecystography, Pyelography, Hysterosaipingography, Phlebography, Myelography, Sialography and examinations of the gastrointestinal tract in monocontrast technique. Tasks requiring low image quality are pelvimetry, many contrast examinations in paediatrics and the location of catheters and tubes. With digital image intensifier radiography the radiation dose for a single image could be lowered for high quality class by 60%, for medium quality class by 85% and for low quality class by up to 95%, as compared to a film/screen exposure (Speed 200). Using TLD measurements of patient and staff doses, examinations of the gastrointestinal tract with digital and analog techniques were compared. These results are currently being processed. Additionally dose maps of different examination rooms were produced. Using these maps members of staff can estimate dose depending on the equipment and their location in the examination room. This study was made for undercouch and overcouch fluoroscopic units with digital and conventional imaging techniques.

Exposure and postprocessing parameters for a new storage phosphor system (Fuji: ACI+) were optimised by comparative studies for bedside imaging in an emergency care unit.

One advantage of digital radiography is the option of evaluating the image quality in digital form. Additional parameters which can be obtained by pixel values include density and dynamic range, noise and signal/noise (S/N) ratio. The S/N ratio is defined as the ratio between mean value and standard deviation in a preselected area. By phantom studies the correlation of dose and image parameters were evaluated. Using the results criteria to determine the optimal dose were described.

A previous study compared the chest image quality of conventional film/screen, asymmetric film/screen, equalisation, storage phosphor and image intensifier techniques by phantom exposures and patient examinations. The quality of 43 patients were classified by seven observers in four different hospitals. According to the results of phantom measurements and a previous study, digital image intensifier radiography was excluded from patient examinations because of its low image quality. The equalisation technique had the best image quality in both mediastinal and peripheral fields of the chest. Compared to film/screen, the asymmetric film/screen was graded higher in the mediastinal field, but lower in the peripheral field. Within this study dose comparative measurements of these five imaging methods with an Alderson phantom were performed in cooperation with the other members of these groups. Dr. Faulkner reports on these results.

The primary goal of our test phantom measurements was the development of effective test procedures for performance characteristics and quality control, which are easy to handle with limited additional equipment, not time consuming and representative of clinical imaging. After two years of study, the following quality assurance procedure for digital image intensifier radiography was recommended.

Frequency: 2 times/month

Testphantoms: DSA-phantom (DIN 6863 - part 8)
Normi (PTW-Freiburg)

Exposure Values: Tube voltage 70kVp
Preselected dose (*) low (20µR)
medium (100µR)
(*) high (500µR)

Entrance field (*) small (14cm)
large (40cm)

Exposure Modes: Single shot
DSA

Parameters for evaluation:

Spatial resolution (video monitor (*), film)
Dynamic range (digital values (*), film-densitometry)
(*) Signal/Noise Ratio (digital values)
Contrast detectability for DSA-mode (monitor(*), film)
(*) Dose values (area dose product, entrance dose)
(*) Documentation of the image/film processing quality by digital stored test images.

(*) To shorten the time for this procedure measurements marked by an asterisk are performed on a monthly basis.

Publications:

1. **Digitale Bildverstarkerradiographie - Welche Aufnahmedosis fur welche Fragestellung? Akt. Radiol. 2 (1992), 111-15.**
   Lehmann KJ, Busch HP, Georgi M.

2. **Thoraxaufnahmen mit dem AMBER-System.**
   Fortschr. Rontgenstr. 156, 3 (1992), 241-246.
   Busch HP, Hartmann J, Freund MC, Lehmann KJ, Georgi M, Richter K.

3. **New Chest Imaging Techniques - A comparison of five analog and digital methods. European Radiology (1992).**
   Busch HP, Lehmann KJ, Drescher P, Georgi M.

4. **An international intercomparision of dose area product meters. Radiation Protection Dosimetry (1992), . 43, No. 1/4, 131-134.**
   Faulkner K, Busch HP, Cooney P, Malone JF, Marshall NM, Rawlings DJ.

5. **Digitale Projektionsradiographie - Teil 2: Klinische Anwendungsmoglichkeiten. Rontgenpraxis 45 (1992), 35-43.**
   Busch, Lehmann KJ, Freund MC, Georgi M.

6. **Digitale Radiographie in der Gastrointestinalen Diagnostik. In: R.W. Gunther, H.P. Gockel: Jahrbuch der Radiologie 1992, Biermann Verlag 1993, 27-35.**
   Georgi M, Busch HP.

7. **Digital Radiography: Quality Assurance and Radiation Protection. Radiologia Diagnostica (1993).**
   Busch HP, Georgi M.

8. **Neue Bildgebende Methoden in der Thorxdiagnostik-Eine Studie zur Evaluation von Speicherfolienradiographie, Schlitztechnik, asymmetrischer und konventioneller Film-Folientechnik Akt. Radiol. 3 (1993), 14-19.**
   Lehmann KJ, Busch HP, Drescher P, Loose R, Georgi M.

9. **Neue analoge und digitale Bildaufnahmeverfahren zur Thorakdiagnostik Prinzip klinische Wertigkeit - Wirtschaftlichkeit.**
   Busch HP, Georgi M.

Progress Report

Contract: FI3P-CT920020 **Sector:** C22

Title: Quality assurance parameters and image quality criteria in computed thomography.

1) Jessen — Univ. Århus - Hospital
2) Ortins — LNETI
3) Schneider — Univ. München

I. Summary of Project Global Objectives and Achievements

The Steady increasing use of computed tomography in adult and paediatric radiology and the fact that recent developments in technology makes this high dose modality even more attractive demand a continual research in performance optimisation and dose reduction. In many clinical situations, it can be necessary to use high values of mAs and thin slices, thus increasing the dose to the patient. In order to obtain an adequate image quality and to ensure optimum radiation protection in computed tomography, it is therefore necessary to have a precise medical indication, to select the examination technique specifically according to the information, which it is wished to obtain, and to minimize the radiation exposure.

This coordinated project is primarily dealing with four subject areas having the objectives described:

A: Image Quality Criteria for adults CT. Testing of image quality criteria and their implementation and further developments of existing criteria formulated by organisations and individuals in order to create a base for more general acceptable criteria. Evaluation of standard protocols for newly installed CT units by assessments of doses and image parameters measured in phantoms.

B: Image Quality Criteria for paediatric CT. Development and testing of image quality criteria and their implementation. Field studies to collect information on technical CT parameters used to perform paediatric examinations. Optimization studies for head and thorax paediatric examinations.

C: Basic research of general image quality parameters in CT. Influence of scanners technical specifications and parameters on patient dose and image quality. Studies of dosimetric methodologies applied in CT.

D: Conventional radiography and fluoroscopy in paediatric . An Europe - wide field study on the 5 year old child, so-called "TLD-3" study. Fluoroscopy - a data exchange on frequent X-ray investigations in children.

The Aarhus group has mainly been concerned by subject areas A and C, the Lisboa group by subject areas B and C and the Munich by B and D. Exchanges among groups of ideas and experience is a very important goal for this coordinated project containing so different structured groups of contractors.

The progress achieved is described in detail by each contractor and the work has been adjusted within the project and with other contractors in the sector Optimisation of Radiation Protection of the Patient at the Contractors Meeting in Brussels April 1993 and further results will be presented at the Grado Workshop in September 1993. A TL intercomparison between contractors has been planned and partly accomplished. It will be finished in the beginning of the next period.

Head of project 1: Dr. K.A. Jessen

II. Objectives for the reporting period.

Work has been undertaken forwards:

- Evaluation of standard protocols for newly installed CT units in Denmark and comparison with the image quality criteria formulated by assessments of doses and image parameters measured in phantoms.

- Further studies of the correlation between dose describtors for computed tomography.

- Testing of image quality criteria and their implementation and further developments of existing criteria for selected CT examination of adults.

Objectives for the next reporting period are following:

- Further testing and developments of image quality criteria extended to other common CT examinations.

- Organ dose and effective dose calculations to evaluate the influence on expected dose reduction for standard protocols.

- Coordination of the project as a whole.

III. Progress achieved including publications.

Measurements of image quality parameters.

The physical parameters[1] for the evaluation of the performances of Computerized Tomography (CT) scanners are: noise, pixel size, spatial resolution, slice width, dose and linearity of CT values.
The physical image quality parameters are defined on the basis of routine tests and have been measured for the standard settings on seven newly installed CT units.

The measurements of CT number stability, resolution and noise have been performed with an RMI (model 461A) combined head/body quality assurance phantom. Resolution (R), noise (S), dose (D) and nominal slice thickness (T) can be combinated to give a Figure of Merit Q[1]. The higher the Q value the better the performance. Results of measurements and calculation are shown in Table 1.

Table 1. Typical parameters for examination of the mediastinum.

Name of Scanner	Scanner- settings			S	R	Q	(CTDI/MAS) *100	(MSAD/MAS) *100	Mean slice number T_n	"Volume" Dose MSAD*T*T_n
	MAS	Slice T	U_{ACC}							
	mAs	mm	kV	%	mm	$mm^{-1} \cdot Gy^{-1/2}$	mGy/mAs	mGy/mAs	—	mGy*cm
1. Somatom Plus S	420	10	120	0,74	1,89	0,82	10,71	9,69	20,7	842,5
2 Somatom Plus S	275	8	137	1,60	1,20	0,83	16,07	14,80	30,4	989,8
3. Somatom HiQ	240	10	133	1,13	—	—	19,52	18,50	34,7	1540,7
4. Picker 2000 PQ	300	10	130	1.17	1,20	0,78	33,83	23,07	46,9	3245,5
5. Tomoscan SR	250	10	120	1,59	1,20	0,79	22,24	14,64	28,5	1043,1
6. Tomoscan LX	332,5	10	120	1,17	1,31	0,85	19,73	13,53	47,7	2146,5
7. Tomoscan LX	332,5	10	120	1,49	1,8	0,41	20,40	13,92	26,1	1208,4

Investigation of the correlation between dose descriptors.

Various dose descriptors are used to define reference dose for CT exposure. The descriptor relating to a single CT slice is the Computed Tomography Dose Index (CTDI)[1]. For multiple slices, which is more like the clinical situation, the Multiple Scan Average Dose (MSAD)[3], Multi-slice[1] and IEC definition[2] are often used as the dose descriptors.

50 dose profiles for a single slice at the center of rotation have been measured free-in-air with TL dosimeters and integrated to give CTDI and Full Width Half Maximum (FWHM). All TL dosimeters have been calibrated in a ^{60}Co radiation field before and after measurements. A correction factor of 0.8 for the quality has been used. The CTDI value based on nominal slice thickness deviates considerably for thin slices of same of the units.

The MSAD values have been measured with the ionisation chamber located at the surface of the phantom of 22 cm in diameter, IEC at 1 cm depth in the phantom and Multi - slice values on the surface of the phantom with TL dosimeters.
A considerable difference between the CTDI value measured free-in-air and the MSAD value has already been demonstrated for some CT-systems[4]. Table 2 illustrates these discrepancies for measurements performed on a Somatom Plus S scanner. The MSAD and IEC values are represented per slices.
A good agreement is obtained for the MSAD, IEC, and Multi-slice dose describtors for this scanner system and a significant deviation to the CTDI describtor measured free-in-air is observed. Even higher descepancies has been registered for systems with shaped filters.

Table 2. Various dose descriptors tested on Somatom Plus S scanner

Free-in-air (TLD)	on the surface of perspex phantom (Capintec PC-4 ionisation chamber)		at 1 cm depth in a perspex phantom (Capintec PC-4 ionisation chamber)			on the surface of perspex phantom (TLD)		
CTDI	MSAD 1 (slice)	MSAD 7 (slices)	IEC 1 (slice)	IEC 7 (slices)	IEC 15 (slices)	Multi slice 7(slices)	Single slice	M/S
mGy	mGy	mGy	mGy	mGy	mGy	mGy	mGy	–
49,1	41,3	40,9	40,5	40,3	39,5	41,4	30,5	1,36

Testing of image quality criteria.

The quality criteria for the images can be based on two anatomical components: the characteristic image features and the critical image elements and on more general "diagnostic" image quality criteria.

For the seven CT units all the scanning parameters, number of slices for each examination etc. have been registered together with relevant information on the patients (size, sex, etc.) within a four weeks period.
At all 1133 examination have been registered. Mediastinum and retroperitoneum have been the biggest two group´s for all hospitals (30,4 %).

A pilot study with a total of 53 examinations (23 in the mediastinum and 30 in the retroperitoneum) had been performed previously on one of the units and has been clinical evaluated by two independent experts according to the criteria formulated recently by the German Federal Chamber of Physician. The criteria predominantly measure anatomic and phatologic features. Criteria related to the basic image quality such as the presence of artefact, disturbing noise and spatial resolution are warranted. It may be desirable to gradate between visualization of structure and sharp reproduction as the CEC criteria for conventional radiography. Therefore modified quality criteria have been formulated for mediastinum and retroperitoneum. These quality criteria will be presented in the Grado workshop.

All the examinations of the two types from the survey period have been evaluated and the score yes/no has been given for the fulfilment of the individual criteria for each examination. The QC factors for all CT systems are shown on Fig.1 as a function of the MSAD value multiplied with the nominal slice thickness in cm and the number of slices. QC is mean of the yes score, given in percentage, of anatomic criteria related

to the pure anatomical image quality. There seems to be no correlation between the quality assessed and the dose.
The same procedure has been used for the retroperitoneum.

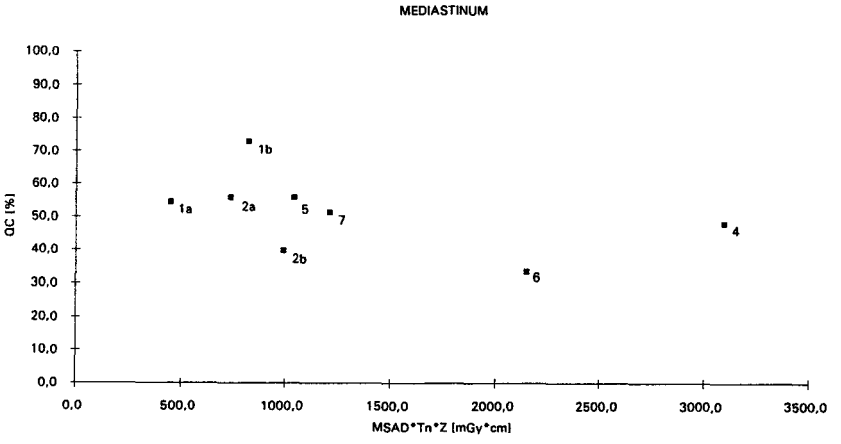


Figure 1. QC factor as a function of " Volume Dose".

On the next phase of this project image quality criteria extended to other common CT examination will be tested and developed.
It will also be investigated relationship between image quality and radiation dose.

Organ doses and effective doses will be calculated for the different examinations on the basis on free-in-air measurements for all CT units. These figures will be used to evaluate the influence on expected dose reduction for standard protocols.

Organ dose calculation will be started in November 1993.

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3. A.F. Carvalho, A. Oliveria, J. Alves, J.V. Carreiro, L.C. Jensen, K.A. Jessen, 1993, *Quality Control in Computed Tomography performed in Portugal and Denmark*, Grado Workshop, September 1993.

Head of project 2: Dr. Ortins

II. Objectives for the reporting period.

Objectives of the first phase of the project were mainly the following:

1. Investigation of basic subjects on dosimetric methodologies applied in CT, and data collection of technical parameters used in paediatric CT examinations;

2. Design and construction of devices and phantoms to be used in the second phase of the project.

Objectives for the next reporting period are the following:

1. Research on dosimetric methodologies applied in CT and the influence of scanner technical specifications and parameters on patient dose and image quality (to be finished until Dec. 93).

2. Optimisation studies for head and thorax paediatric examinations (Oct. 93 to April 94).

3. Field studies and testing of image quality criteria (Oct. 93 - Jun. 94).

III. Progress achieved, including publications.

Dosimetric methodologies applied in CT.

The accuracy of CT doses evaluated can be affected by dose descriptors, scanner technical specifications, experimental setup and its relation with dimension and position of patient body (or phantom) into the radiation beam.

1. The most frequently used dose descriptor is the Computed Tomography Dose Index (*CTDI*), defined by $(\Sigma D_i x_i) / T$, where D_i is the dose reading of the dosimeter i with width x_i and T is the nominal slice thickness. Besides it is known that for nearly all scanners the nominal value of slice thickness (T) does not coincide with the actual value defined as the full width at half maximum

(*FWHM*) of the dose profile. Therefore, *CTDI* values are systematically under or over estimated.

To investigate the importance of this systematic inaccuracy, it was developed a mathematical function and a software package to calculate profile parameters - symmetry, asymmetry, left and right penumbra, length of the plateau and full width at half maximum - from the dose readings obtained from a set of TLD's irradiated free-in-air in scanner isocentre.

Then, a more precise dose descriptor ($CTDI^*$) could be defined in routine basis, replacing in the definition of *CTDI* the nominal slice thickness (T) by the actual value of *FWHM* . In figure (1) dose profile and both descriptors *CTDI* and $CTDI^*$ are drawn and the physical meaning of $CTDI^*$ can be clearly understood.

From data of TLD measurements, *CTDI* , $CTDI^*$ and plateau dose are compared on figures (2) , (3) and (4).

A complete report will be presented in Grado workshop(1).

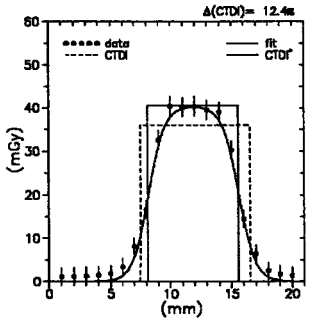
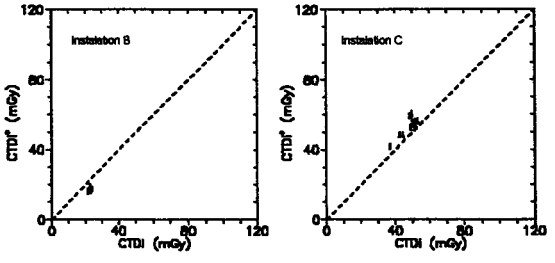


Figure 1 - Dose profile and comparison between CTDI and $CTDI^*$.



Figures 2 e 3 - Comparison of CTDI and $CTDI^*$ in two installations.

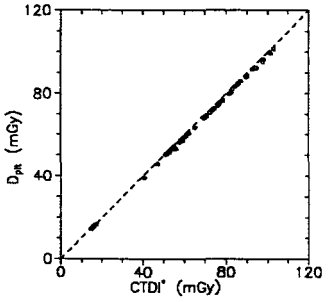


Figure 4. - CTDI* versus plateau dose.

2. Theoretical work was carried out to establish mathematical relations:

   - between isocentre doses and phantom surface doses;

   - to describe dose values changes when central line of patient body is not crossing the scanner isocentre.

   Experimental work will be carried out until Dec. 93 to validate these theoretical work.

Field studies to collect information on technical CT parameters used to perform paediatric examinations.

CT examinations are being performed with a very large range of technical parameters and doses. For certain type of examination the use of a set of parameters appears in some cases to be related with recommendations from manufacturer of scanner, in other cases with radiologist or technician training and it was demonstrated by us before, that in paediatric head examination doses can be reduced frequently by 50% to 75% without lost of clinical information required to diagnosis.

Therefore, to test in field "quality criteria for CT in paediatrics" developed in project 3 (Dr. K. Shneider, Univ. Munich), will be necessary to know for each CT installation: equipment available, frequency of paediatric examination, staff training. It was prepared a questionnaire form with three parts:

- Scanner manufacturer, model and year; multiformat chamber manufacturer, model and year; service periodicity; frequency of paediatrics and adults CT examinations.

- Brain examination.

  CT technique and parameters used per age group (0-1; 1-5; 5-15 years), characteristic image details; critical image details.

- Thorax examination.

  CT technique and parameters used per age group; characteristics image details; critical image details.

Field study will start in Oct. 93.

Quality Control in CT.

It was analysed the data collected in a programme of QC carried out in the previous CCE contract period. Results will be presented together with Arhus University at Grado Workshop(2).

Intercomparision of dose measurements.

Participation in a intercomparision of a dose measurements carried out in CT and cobalt radiation beams. Results are presented by project coordenator.

Publications

(1) - Dose Profile and Dose Index Analysis in Computed Tomography, A D Oliveira, J G Alves, A F Carvalho and J V Carreiro (to be presented in Grado workshop).

(2) - Quality Control in Computed Tomography performed in Portugal and Denmark (to be presented in Grado Workshop).

Head of project 3: Dr. Schneider

II. Objectives for the reporting period

1. CT-project (children)
1.1. Pilot study in clinics and doctor's offices (Southern Germany)
1.1.1. Information on the equipment
1.1.2. Dose measurements with thermoluminescence dosemeters 'free-in-air' for the corresponding equipment settings
1.1.3. First results

1.2. Measurements to get the distribution of dose in the gantry

1.3. Preparation of phantom measurements

1.4. Development of criteria on computed tomography in pediatrics

2. TLD III - project (dose measurements - five years old child)
2.1. Preparation of a participation list; call for participation
2.2. Mailing of dosemeters and questionnaires; first returns of completed questionnaires and X-ray films
2.3. Organization of film-reading in November

III. Progress achieved including publications

ad 1. As a basis for the planned recommended guidelines in paediatric CT, it is necessary to determine the limits of dose reduction which still allow a diagnostically sufficient image quality. The determination of radiation exposure requires current data on the types of CT examinations of children with their respective doses.

ad 1.1 Therefore a pilot study was developed to define guidelines for CT examinations with the goal of reducing radiation exposure and optimising image quality for examinations on infants and children.

Twenty-two clinics and 39 doctor's offices with CT-units in the south of Germany were asked to participate in this study. Nineteen offices and 21 clinics were willing to take part in this study. Dose measurements were made for 43 CT-units (2 offices and one clinic had 2 CT-units).

The survey in the greater area of Munich (Southern Bavaria: 20 CT-units in 9 clinics and 10 offices) was conducted by the research group of the Children's Clinic of the Ludwig-Maximilians-Universität München (Dr. Schneider) in cooperation with the GSF Neuherberg (Mr. Panzer).

The survey in Middle and Northern Bavaria (23 CT-units in 12 clinics and 9 offices) was made by the research group of the Children's Clinic University of Würzburg (Dr. Horwitz) in cooperation with the Medical Physics Dept. of the Clinic for Radiation Therapy of the University of Würzburg (Prof. Richter).

ad 1.1.1. A questionnaire surveyed information on the CT-unit,
- manufacturer, construction year, detector type, focus size;
- documentation type (analog- or laser camera);
- details on maintenance including quality and constancy control of the equipment;
- total number of examined patients and proportion of examinations in children
- information on beam filtration, slice thickness and parameter settings (kV, mAs)

ad 1.1.2. The TLDs provided by the GSF were placed in either polyvinylchloride (Munich) or plexiglass (Würzburg) capsules which were fastened to a stand which has to be placed in the centre of the gantry.

The TLDs measured the dose for one slice 'free-in-air' using the clinic/office specific settings for the respective body region (skull, chest, abdomen and spine). To calculate the dose, information on the filtration, slice thickness and parameter settings (kV, mAs) was needed.

Based on these measurements ('dose free-in-air' for each slice in the centre of the gantry), a calculation of organ dose using a special mathematical method ("Monte-Carlo-Method") will be made.

ad 1.1.3. With the exception of one CT-unit from the Picker company, all units were of the third generation — rotating scanners with a moving detector system and detector banana. Picker is the only manufacturer producing CTs of the fourth generation — fixed 360° detector circle and moving tube.

The most frequent CT-unit manufacturers were Siemens, followed by General Electric, Picker, Toshiba, Elscint and Philips. The oldest unit was constructed in 1983; a total of five units were older than five years. In the offices, no CT-unit was older than two years. The documentation was nearly always done by a laser camera.

Additional controls for quality assurance tests were made by only one participant. Maintenance and constancy control were done by the technician of the corresponding firm. Only one office had no maintenance contract for its unit.

The dose measurements on all CT-units are completed. Evaluation and analysis of the data are in progress.

ad 1.2. Additional measurements for the face skull and special high resolution examinations settings and measurements of dose for variations in the parameter settings, such as kV and/or mAs and slice thickness for different TLD positions off from the centre of the gantry were made by the Würzburg research group. These "displacements" correspond to the neighbouring regions along the body axis, or for a lateral offset, to incorrect positioning of the patient. Respective measurements were also made for settings for a spiral technique.

ad 1.3. A phantom is necessary to gather exact data on the relationship between image quality and radiation dose. Therefore, one further goal of this project is to develop such an appropriate CT-phantom. Data on image quality and dose will be gathered using this body phantom for various settings. The phantom will also provide information on the dose distribution within the body.

ad 1.4. The task to define CT image criteria for the different body regions has been assigned to the different members of the "Lake Starnberg Group". The preliminary definitions will be collected by the meeting of this group in November where they will be discussed.

ad 2. Similar to the previous two TLD surveys in infants, dose measurements for a five year old child for conventional X-ray examinations are in field. A total of 121 clinics in 19 European countries have been asked to participate. In addition to the children's clinics of the member states of the European Community, clinics from Switzerland, Austria, Scandinavia and other East-European countries have been included in this survey.

The revision of the questionnaires, data collection and data analysis is being done by the research group of the Children's Clinic of the University of München. Dosemeter readings are being made by GSF, Neuherberg (D) and NRPB, Chilton (UK), USL Nº 7, Udine (I). Middle and Eastern Europe has been assigned to the GSF, Northern Europe and Scandinavia to Chilton and Southern Europe and Czech. Rep. to Udine.

Distribution of the TLD-measurements in the three European centres of Medical Physics

Chilton	UK	: 12	Udine	France	: 9
	Rep.of Ireland	: 2		Italy	: 6
	Sweden	: 3		Spain	: 8
	Iceland	: 1		Greece	: 3
	Finland	: 3		Czech Rep.	: 1
	Norway	: 3		Portugal	: 1

GSF	Germany	: 46
	Netherlands	: 2
	Austria	: 4
	Switzerland	: 5
	Belgium	: 8
	Poland	: 1
	Denmark	: 1
	Hungary	: 2

The following X-ray examinations are surveyed in the five year old child:

Chest ap/pa & lateral
Skull ap & lateral
Pelvis ap
Abdomen ap
Intravenous pyelogram (IVP)

Each clinic received from the respective mailing centre 10 TLDs and a set of questionnaires on equipment, technical parameter settings (kV, mAs, dose-area product etc.) and radiation protection measure.

Mailing started at the end of May. The X-ray films will be evaluated by the "Lake Starnberg Group" in November 1993.

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Teil I: Thoraxaufnahmen.
Zbl.Rad.(in press)

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Zbl.Rad.(in press)

Progress Report

Contract: **FI3P-CT920024** **Sector: C22**

Title: Diagnosis related dose: an investigation on patient risk and image quality in european hospitals.

1) Van Loon Univ. Bruxelles (VUB)
2) Thijssen Univ. Nijmegen

I. Summary of Project Global Objectives and Achievements

The main sources of low dose radiation to the population of Europe are the medical diagnostic examinations in radiology and nuclear medicine. The amount of radiation given before a certain diagnosis is made, depends on factors of medical, technical and/or organizing character.

- In the medical sector we identify the justification of an examination and the protocol leading to a diagnosis. Little work is done on the influence on the dose by these items so far.
- In the technical sector many variables are identified and great effort has been devoted to evaluate the effects of quality assurance and to establish guide-lines for the improvement of image quality and the reduction of patient dose.
- In the organizing sector many extrinsic and logistic factors -e.g. factors that are linked to the differences in the organization of the health services- can compete with an optimum cost/benefit relation.

No effort has been made to relate the dose and therefore the risk to establish a correct diagnosis in different European Hospitals, to those three factors. One method that can contribute to a better understanding of this relationship is the concept of Diagnostic Groups (DGs): a DG is the set of examinations that has led to one and the same diagnosis.

The pilot study "Diagnosis Related Dose" [1] showed that a DG can be established and clearly described -at least for some diagnoses- and that the elements determining the dose can be retrieved. A first draft of a methodology for patient data and equipment data collection is available from this study.

The goal of the underlying project is to use this method in more hospitals, to develop the use of the Image Quality Figure[2] and the dose, to rank different strategies mathematically, and inventoriate and discuss the reasons that can lead to the choice of examinations and number of images and finally evaluate the medical and/or sociological data.

Economic and technical benefits expected are:

- Contribution to the elaboration of guidelines for good radiological practice, which can lead to a lower exposure to radiation of the European population.
- Supply of information useful to governmental and European bodies about extrinsic and logistic factors that influence the dose to patients. This may lead to a better use of health resources.

The objectives can be summarised in following items:

- Exploitation of the results obtained in the pilot study, and refine some approaches;
- Completion of the data collected by data from partners in Madrid, Udine and Paris (Subcontractors 03,04,05);
- Calculation of absorbed equivalent dose from all used imaging techniques, as far it can be done with the help of the available literature data;

- Use of the Image Quality Figure (IQF) [2] and the dose to rank different diagnostic strategies mathematically;
- Inventory of reasons that can lead to the choice of examinations and number of images and evaluate the medical, sociological and organization factors involved, and their impact on the patient dose;
-Describing the methodology of the DGs in a publication so that the dose and risk evaluation can be performed by other institutes.

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Head of project 1: Prof. Van Loon

II Objectives for the reporting period

1. - Design and evaluation of data forms for the collection of patient data and examination-procedure data; draw up of an accompanying explanatory document, to allow the data collection to be carried out by remote teams.
2. - Collection of medical data in the three subcontractors' centres (in Madrid, Udine and Paris), updating of the data sets in Brussels.
3. - Setup of the database system to handle the collected data.
4. - Elaboration of the protocol for dosimetry and image quality (in collaboration with the Nijmegen team).
5. - Testing, in collaboration with the team in Nijmegen, of a set of test objects for the dosimetric field measurements in the partners' hospitals.
6. - Start of the field measurements of doses and image quality in the centres in Madrid, Udine and Paris .

III Objectives for the future

- Further development of the database structure.
- Performing the remaining measurements of physical parameters in the three subcontractors' institutes.
- Assessment of the doses per patient in the different institutes for both DGs.
- Evaluation of the method.
- Publication of the results and a practical guide for the method used.

IV Progress achieved

1. Data Forms.

Based on the experience gained in the pilot project a series of forms was designed.
For the collection of the patient data a form was developed to list all the examinations for a single patient. A series of 8 forms was designed to collect the technical modalities used for the examinations and four forms for the technical information about the equipment used.
Evaluation of the validity of the forms was done by the teams of Nijmegen and Brussels.
In order to prevent ambiguous data collection, an accompanying document explained the limitations of the data to be collected.

2. Data.

Using these forms, data collection was started by the three subcontractors.
Presently data were already received from:
Madrid: (Prof. E. Vaño) Renal Cell Carcinoma and Lumbar Hernia Discalis
France: (Dr C.Maccia) Renal Cell Carcinoma (these data were collected at the "Hôpital Sud" in Amiens)
The data from the Udine team (Prof. R. Padovani) and those from Paris for Lumbar Hernia Discalis DG will be available at the end of November 1993.
Using the new data forms, the patient and examination data for the two DGs in the Brussels Hospital were completed.

3. Database.

To handle and process all the information received, a database is developed based on the Microsoft®Excel 4.0 programme. By this choice, macro programming makes rapid access to the information available.
An example of the output of the database is a plot of the frequencies of examinations per patient in four centres for the Renal Cell Carcinoma DG. It is clearly seen that there are important differences in the choice of examination types between the centres to achieve the same diagnose. The -preliminary- results of the second DG show a comparable pattern. These results affirm the results of the pilot study.

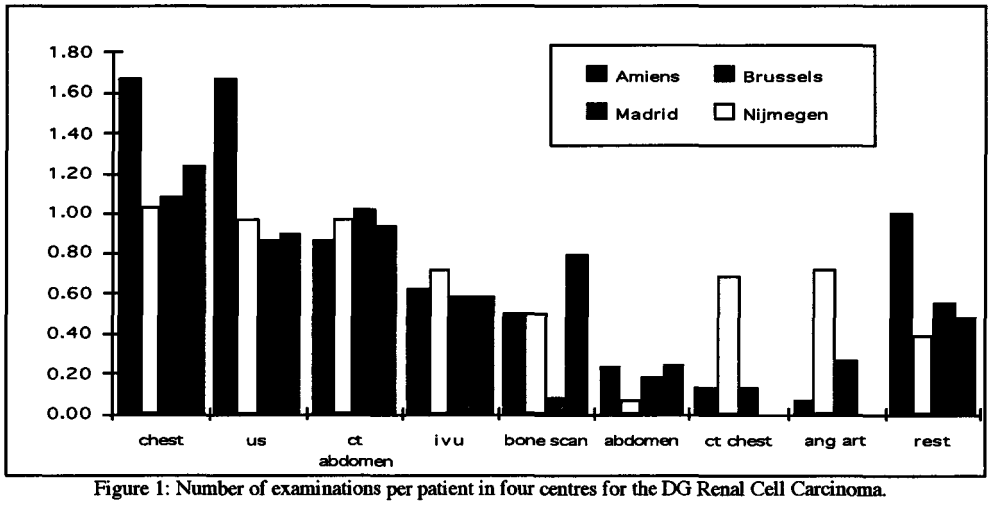


Figure 1: Number of examinations per patient in four centres for the DG Renal Cell Carcinoma.

4. Dosimetry.

A protocol for dosimetric measurements is defined to investigate the dosimetric consequences of differences in the diagnostic paths. At the development of this protocol, the following considerations were taken into account:

- To be able to assess the cumulative effect from radiological techniques with very different dose distributions in the patient, the effective dose as defined by the ICRP report 60 is chosen as the dose descriptor.

- It must be possible to compare dosimetric results obtained during this investigation with those collected by other teams. Entrance surface dose is measured for the non-CT examinations, and is defined as the dose to air at the point of intersection of the X-ray beam axis with the entrance surface of the patient, including backscattered radiation[3]. For Computed Tomography, CTDI-index was measured for each slice thickness used in clinical routine. The dosimetry protocols used are based on the NRPB reports 186 (radiography) [4] and 249 (CT) [5]. The measurements are performed with LiF TLDs.

- To be independent of patient geometry, dose measurements are carried out on test objects: a cylindrical phantom for CT, a flat perspex-aluminium phantom for the other examinations (see § 5).

- To correct dose measurements for the energy spectrum of the beam used, high voltage and beam filtration are measured at all equipments involved in the patient examinations.

Image Quality.

For Radiography equipment a Contrast Detail test object is used which allows us to introduce the Image Quality Figure for a quantification of the image quality. The CD test object is used as the central PMMA plate in the test object mentioned in table 1. The image quality is measured under standardized conditions i.e. at an effective tube potential of 125 kVp for chest radiography and 75 kVp for other radiological techniques, and a mAs leading to a net film density of 1.0.
For Angiography equipment the image quality is also measured using the CD test object. In the case of DSA, one set of images is filmed with a plain test object according to the projection simulated and another set with the central PMMA plate replaced by the CD test object. The two sets of images are then subtracted, and the image is evaluated at the monitor.
For Computed Tomography the image quality is evaluated using three parameters: The image noise is defined as the standard deviation of the Houndsfield Units in a homogeneous water volume, the dose is measured as the CTDI in air in the centre of the gantry and the resolution is determined from the image of a thin wire. These parameters can, in the future, be combined to the figure of merit Q[6]. These data can then be compared with those published by Jessen[7].
The protocol was tested in Nijmegen by the Nijmegen and Brussels teams.

5. Test objects.

Based on the results of the pilot project, a new set of test objects was developed for the most important radiographic views. (See report project 2, M A O Thijssen, Nijmegen) The test objects consist of PMMA and aluminium plates of 30 x 30 cm and a total thickness depending on the radiographic view. The different compilations are given in Table 1.

Radiographic view	cm PMMA	mm Al
Chest AP/PA	9	0
Chest LAT	19	0
Lumbar Spine AP	19	8
Lumbar Spine LAT	19	20
Abdomen AP	19	8 spine 0 soft tissue
Pelvis AP	19	2

Table 1: Thicknesses of PMMA and aluminium in test objects for different radiological views.

6. Measurements.

A first set of measurements was performed on the equipment in the 'Hôpital Sud' in Amiens, France. The protocol described has proved to be well applicable. Similar measurements will be performed in Madrid (second half of October), Udine (end of November) and Paris (first half of December).

References

3 National Protocol for Patient Dose Measurements in Diagnostic Radiology, Working Party of the Institude of Physical Sciences in Medicine, 1993

4 DG Jones, BF Wall, Organ Doses from Medical X-ray Examinations Calculated Using Monte Carlo Techniques,1985, NRPB Report **186**

5 DG Jones, PC Shrimpton, Survey of CT Practice in the UK; Part 3: Normalised Organ Doses Calculated using Monte Carlo Techniques, 1991 NRPB Report **250**

6 HPA (1981); Measurement of the performance characteristics of Diagnostic X-Ray systems Used in Medicine, Part III Computed Tomograpgy X-ray Scanners; HPA Topic Group Report 32.

7 KA Jessen, J Jørgensen (1989); Quality Control in Quantitative Computed Tomography; British Institute of Radiology, Report **18**; p84-86, London

Head of project 2: Dr. M.A.O. Thijssen

II Objectives reporting period

1. Collection of data on the number and size of x-ray images for several x-ray techniques.
2. Development of test objects corresponding to different parts of the human body.
3. Development of a measuring protocol for the skin dose of a standard patient for all equipments used. The results must be directly comparable with other published data.
4. The quantification of image quality in terms of the Image Quality Figure (IQF) of the images of which the dose is measured.
5. Calculation of absorbed dose from skin dose.
6. Starting of the field measurements of dose and image quality in the centres in Paris, Amiens, Madrid and Udine.

III Objectives for the future

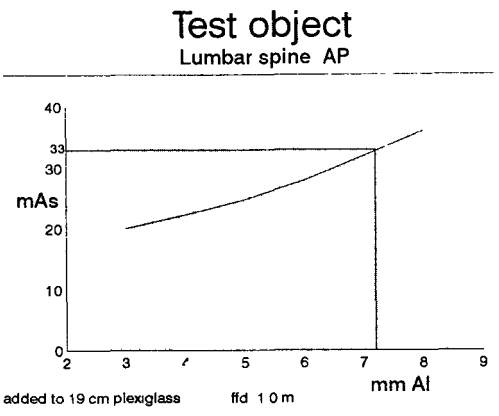
1. Further study of a method to calculate dose equivalent and radiation risk per examination, from the absorbed dose.
2. Complete the measurements of dose and image quality in the new participating hospitals.
3. Contribution to the comparison, the interpretation and the final report.

IV Progress achieved

1.
Because of a shift in the time interval that was established for the dates of the examinations since our pilot study, additional patient data files were collected. Out of this files information was retrieved about the particular performance of the examinations involved.

2.
In order to standardize the dose measurements, test objects were developed for different parts of the human body. Since simplicity and reproducibility are of importance, the test objects were chosen to be made of plexiglass and aluminium. Plexiglass for the soft tissue and aluminium for the bone.To find the right composition of the two materials, corresponding to lumbar spine, pelvis and chest of a patient of medium size and about 70 kg, data about kVp, mAs and thickness were collected from real examinations in Nijmegen as well as in Brussels. After gathering this information, combinations of varying thicknesses of plexiglass and aluminium were used as test objects. Since for every combination the mAs was measured, it was possible to retrieve the correct equivalent of the different parts of the human body in the form of plexiglass and aluminium.

3.
During the diagnostic procedure, different techniques of examination were performed; plain film x-ray, computed tomography, angiography and nuclear medicine. For all these techniques specific approaches had to be developed to determine dose and image quality. For plain x-ray, computed tomography and angiography TLD s-100 were used for dose measurements. A set of three chips LiF:Mg:Ti was put on the tube side of the test object. To find the dose involved with CT-examinations the CTDI was measured. (An axial dose measurement in air using TLD s, as defined by the NRPB report 249).
For the nuclear medicine examinations the dose equivalent was retrieved from literature, after gathering the information about the pharmaceutical that was used.



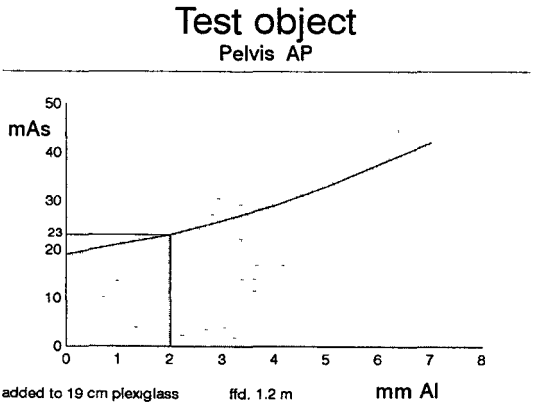


fig. 1a and 1b : Test objects for several parts of the human body were developed, using realistic mAs values from real examinations on patients of medium size and about 70 kg. Plexiglass for soft tissue, aluminium for the bone.

4.
To determine the image quality for plain x-ray and angiography the Contrast Detail phantom was used. For Angiography the Contrast Detail phantom was filmed a few seconds. The results were examined on the monitor of angiography equipment.
To find a measure of the image quality for CT, the noise, and line spread function were determined. For the technique of Nuclear Medicine the image quality was not taken into account.

5.
As a possibility to retrieve the dose equivalent from dose measurements for Computed Tomography, the tables for Dose Calculation from the 'Institut für Strahlenschutz', M. Zankl, W. Panzer and G. Drexler were proposed.

6.
In cooperation with the V.U.B. hospital, measurements were done in the hospital in Amiens, France; Hôpital Sud. Methods for dose measurements and determination of image quality as developed in Nijmegen and Brussels were proved to be suitable in other institutions. Only the determination of the characteristics of a line spread function in a CT image, appeared not to be possible on every CT equipment. Therefore a method of equal value was developed, using the Houndsfield Units of the pixels separately.
Measurements in the remaining countries are expected to find place within a few weeks.

Reference :
M. Zankl, W. Panzer, G. Drexler, 1991
"The Calculation of Dose from External Photon Exposures using Reference Human Phantoms and Monte Carlo Methods" Part VI: Organ Doses from Computed Tomography Examinations, GSF - Report 30/91.

Progress Report

Contract: FI3P-CT920037 **Sector:** C22

Title: Optimisation of image quality and reduction of patient exposure in medical diagnostic radiology.

1)	Maccia	CAATS
2)	Moores	IRS Ltd.
4)	Dance	Hosp. Royal Marsden
5)	Padovani	Unitá Sanitaria Locale - Udine
6)	Vaño Carruana	Univ. Madrid - Complutense

I. Summary of Project Global Objectives and Achievements

Two main observations have emerged from the work carried out during the reporting period :

• the laboratory involved in theoretical aspects of computing codes and mathematical simulation (project n° 4) began to study the techniques necessary to include patient inhomogeneities within the model which can be used for the optimisation of anti-scatter grid design in diagnostic radiology.
The close collaboration established by this group with the University of Linköping (not yet officially included in this coordinated project) was mantained and ensured the achievement of the objectives of the reporting period.

• the scientific work started within the framework of the previous coordinated project concerning the design of an Expert System prototype for Quality Control in mammography successfully continued through an effective collaboration among three laboratories mainly involved in the project (project n° 1, 5 and 6).
As far as the project 2 is concerned, an interesting work was carried out on the relevance of test phantoms employed in mammography as a tool for diagnosing possible changes in the technical performance of radiographic systems.

In conclusion all laboratories included in the coordinated project have made considerable efforts in order to respect and achieve their own project objectives.

Head of project 1 : Dr. Maccia

II. Objectives for the reporting period

The principal objectives for the reporting period were (1) to participate to the improvement of the existing Expert System for Quality control prototype developed in collaboration with the Udine laboratory in Italy and the University of Medicine in Madrid (Spain) (2) to contribute to the validation of the concept of quality criteria for radiographic images listed in the CEC working document issued in June 1990.

III. Progress report achieved including publications

Part 1 : Expert system for Quality Control in mammography.

Data collected through a permanent Quality Control survey of 55 mammographic equipement used within the context of the Bas Rhin breast cancer screening initiative were analyzed in view of establishing relantionships between x-ray film processors' parameter variations and image quality (gross fog, speed index, contrast). The most common types of mammographic films made by different firms (Kodak, Agfa, Fuji, 3M, Dupont etc.) were found to be used in such a screening campaign and several models of processors were checked according to the frequencies of the tests (sensitometry) included in the designed Quality Control protocol. This enabled us to collect daily information on sensitometric strips processed in the most active screening centres (about 70% of the total screening activity was thus included) over a period of two years.

Large variations in the parameters of the mammography film which characterise the state of the process were observed among the controlled centres (the extreme contrast values for all film types ranged from 1.7 to 4.3 ; the speed of the films ranged from 470 to 550 (these speed values are normalized on the basis of the speed of KODAK 'X-Omat' S film being defined as 500) ; maximum optical density ranged from 3.15 to 4.40).

A fairly comprehensive database was therefore created for each processing equipment. A much deeper data analysis is being initiated for different sets of processors to check the consistancy of the corrective actions already taken to improve the 'on-site' situation. This analysis will constitute an element of the log-book which will be implemented in the knowledge-base of the expert system prototype disegned in Udine. It is already anticipated that this work, which is reporting on the actual performances of a relatively small number of installations, will require in the future much closer collaboration with the industry (research and development departments of film makers in particular) in

order to be able to provide more exaustive and pertinent information to the Expert System prototype.

Part 2 : Image Quality criteria.

The work carried out in this field was mainly oriented toward the organisation, the collection and the interpretation of the data gathered during the second CEC trial on quality criteria of radiographic images.

In order to validate and demonstrate the usefulness of the CEC working document issued in june 1990 on a much broader scale, a second Trial was carried out in 1991, specifically for chest, lumbar spine and breast examinations. The overall framework relating to the organisation of data collection was the following : three different questionnaires, one for each type of examination, were prepared and sent out by the CEC services to the national level coordinators in various European countries who previously had established contact with local X-ray departments. At the same time, three european dosimetry laboratories, namely, the (GSF) in Germany, the (NRPB) in U.K. and the (USL n° 7) in Italy, sent thermoluminescent dosimeters (TLD) to all participating X-ray departments. These TLDs were to be used for measuring, for each projection type and for each patient, the entrance dose received during the examination.

For each examination, the following data were gathered : image criteria, type of X-ray equipment used, filtration, focal spot sizes, AEC, type of grid, type of film, cassette and film processor, patient thickness, kVp, FFD, film size, exposure time, patient dose, mA, and speed class of film-screen combination.

Once the field survey was completed, all information, including the questionnaires, the original X-ray films and the readout of the TLDs were centralized at the (CAATS) in France for evaluation.

In order to provide an independant analysis of the image quality of these examinations for comparative purposes, a group of 15 european expert radiologists met in Paris for one week. Five experts were assigned to each examination type. Their mission was to review all collected films using the same quality criteria and questionnaires as were used by the field radiologists. Table 1 gives a quantitative overview of the size of the project and of the scope of the work performed. Almost all western European countries, as well as one Southern American country, participated in this initiative. More than 80 radiological departments provided approximately 2,000 X-ray films which were checked by the 15 expert radiologists. Three laboratories provided (TLDs) to each participating hospital for measuring the patient entrance skin dose received during the examinations. Each laboratory also read out the 700 doses from the TLDs it had distributed.

NUMBER OF COUNTRIES :	16
NUMBER OF X-RAY DEPARTMENTS :	83
NUMBER OF PATIENTS :	1,108
NUMBER OF FILMS :	2,088
NUMBER OF DOSE MEASUREMENTS :	2,136
NUMBER OF EXPERT RADIOLOGISTS :	15
NUMBER OF DOSIMETRY LABORATORIES :	3

Table 1 : General information describing the CEC Trial.

General Results for Patient Dose

Table 2 indicates the number of dose measurements actually carried out, together with their corresponding statistical range (minimum, mean, third quartile and maximum values), by type of X-ray projection. This table also includes the reference values given in the CEC quality criteria document.

Depending on the type of examination considered, ratios between maximum and minimum dose values ranged from 40 (breast cranio-caudal projection) to 400 (chest PA projection). Wide distribution of doses were found. Table 2 permits a comparison between third quartile dose values and their corresponding CEC reference doses. In general, Trial results seem to be consistent with CEC references, except for breast examinations.

	CHEST		BREAST		LUMBAR SPINE		
Type of Projection	PA	Lat	CC	Lat	AP or PA	Lat	L5-S1
Number of measurements	354	257	369	408	301	308	139
Minimum	0.03	0.13	1	0.5	0.5	0.9	2.7
Mean ± 1 S.D.	0.36±0.75	1.31±2.9	7.8±5.4	9.2±7.3	7.8±6.8	18.7±13.3	34.6±20.3
Maximum	11.99	37.94	41.8	54.5	63.9	68.5	119.9
Third quartile	0.39	1.22	10.04	11.99	9.99	26.49	45.7
CEC reference	0.3	1.5	7	7	10	30	40

Table 2 : Dose statistics by examination and projection type (mGy).

As far the participating hospitals are concerned, more than 70% of hospitals' mean dose values were in compliance with CEC dose requirements for chest and lumbar spine examinations. It was more difficult to respect such levels for breast examinations; only about 40% of doses were in the acceptable range.

General Results for Image Criteria

As mentioned above, field radiologists opinions on image quality were compared to those given by the experts. Each expert worked independently on a seperate new view box and scored films using the same type of questionnaires and the same scoring system as had been used by the field radiologists (0 = no ; 1 = yes). Thus, each film received six readings in all, five from the experts and one previously from the field radiologist. In order to test the inter-rater reliability of the experts, comparisons were made between their five readings of each projection. Conclusions were drawn for the criteria on which the experts disagreed to determine which factor of reliability was involved : explicitness of definition of the criterion, or the feasibility of applying it.

Conclusions

The results have shown that the set of criteria defined for evaluating the quality of diagnostic films for the Breast, Chest, and Lumbar Spine examinations can be applied consistently by both field radiologists and international experts. These criteria represent an improvement in determining film acceptability over the subjective definitions of individual radiologists acting alone.

The following detailed observations can be made : **a)** Harmonisation of practice is necessary since radiographic practices vary excessively for several parameters. **b)** Training of radiographers is crucial for optimising current operating condition of their equipment. **c)** Patient dose varies markedly for all projection types. However, more than 50% percent of hospital mean doses respect the CEC reference dose value. **d)** No statistically significant correlation was found between patient dose and image quality. **e)** Seven image criteria out of 29 failed to meet validation requirements for inter-rater reliability. Despite these minor problems, the image quality criteria system was shown to be acceptable and applicable by both the field radiologists and the international experts. After minor improvement, they may be used to optimise image quality in these three diagnostic radiological examinations, while avoiding unnecessarily high radiation doses to patients.

References

C. Maccia, M. Ariche-Cohen, C. Severo, X. Nadeau. The 1991 Trial on Quality Criteria for radiographic images CEC draft report XII/221/93.

C. Maccia, R. Renaud, S. Castellano, P. Schaffer, R. Whal, P. Haehnel, G. Dale, B. Gairard. Quality control in mammography : an initiative in France. (to be published in the BJR)

Head of project 2: Dr. Moores

II. Objectives for the reporting period

2.1 To assess the relationship between test phantom measurements and the radiographic factors employed.

2.2 To highlight local circumstances which dictate imaging performance as measured by test phantoms.

2.3 To investigate factors which influence the transportability of expert systems.

III. Progress achieved including publications

3.1 Progress achieved.

Image quality in mammography is dictated by the technical factors employed and the operators ability to perform the examination. Having once defined the desired, level of image quality the combination of technical or radiographic factors which should be employed to achieve this level can be specified. Quality control programmes then attempt to verify that the desired radiographic factors are indeed being employed routinely and, therefore, the technical requirements for image quality are fulfilled.

Test phantoms are employed in mammography to verify the efficacy of quality control programmes by demonstrating that a particular level of image quality is routinely achieved. Such phantoms normally assess image quality in terms of high contrast detectability small and large detail threshold contrast detectability and dose delivered. The possibility of utilising such phantoms to diagnose possible changes in the technical performance of radiographic systems forms the basis of the expert system approach presently under development. Unfortunately, to date, there has been no clear detailed analysis of the multi parameter variations which relate technical factors to resolution or threshold contrast imaging performance as measured from test phantom images.

The factors which are known to affect resolution, measured from a test phantom image, are:

1. Focal spot size.

2. Focus to film and test phantom to film distances.

3. Screen/film combination design and processing conditions.

4. Grid.

5. Beam quality including kVp.

6. Screen/film contact including presence of dust and time after cassette loading of film.

7. Film density.

8. Viewer and viewing conditions.

9. Phantom design.

Large area threshold contrast detectability is known to be affected by factors 2, 3, 4, 5, 6, 7, 8 and 9 and small area contrast detectability by all of them, but to a different degree than the high contrast resolution measurement. In some instances the effect may be greater and in others less.

The effect of many of the above factors have been studied previously. However, the exact frequency and magnitude of these effects on a universal scale has not been evaluated, nor have the potential inter-relationships between them. All of these need to be considered when attempting to develop a basis for fault prediction inherent in an expert system approach since they affect the accuracy of predictive values. This accuracy fundamentally affects the relevance of advice provided by an expert system in terms of predicting the possible causes for changes in imaging performance assessed from a test phantom image. If the predictive uncertainty on any cause exceeds the difference in mean values for different causes, then the advice provided by an expert system will be statistically inadequate.

Similarly, if the base-line technical conditions under which an x-ray image is produced, e.g. kVp, focal spot size, screen/film combination, etc. define or are related to the most likely causes of change in performance, then the predictive advice provided by an expert system will need to accommodate local circumstances. Such a situation would seriously affect the transportability of any expert system since local and varied programming would be required.

A number of studies of factors affecting test phantom images have been performed and these have been reviewed. In the case of high contrast resolution, both our own and the work of Gais et al (1984) clearly demonstrate the effects of inter-observer variations on measured resolutions as determined by test grid patterns. Figure 1

presents the results of Gais et al and indicates a mean inter-observer variation on visually determined resolution limits of 0.33 lp/mm. These results are in agreement with results presented by ourselves in our interim report to the Commission's Radiation Protection Research Programme for the period 1990-91. In fact, much of the work presented at the Würzburg Workshop on Test Phantoms and Optimisation in Diagnostic Radiology and Nuclear Medicine in June 1992, and organised in conjunction with the Commission, highlighted the significant effect of inter-observer variations which can occur generally when interpreting test phantom images.

The effect of screen/film combinations and processing conditions have been investigated [Law and Kirkpatrick (1988, 1989) DHSS (1991)]. Figure 2 presents measured contrast-detail curves measured in the UK [DHSS (1991)] indicating the wide variations in performance actually observed. When this data is combined with the effects of variations in processing conditions which will exist in practice, the contrast-detail performance to be expected will be extremely blurred.

The accuracy of and method by which the technical factors, which are employed radiographically, are assessed will also affect the predictive certainty. These factors themselves can only be assessed to a particular level of accuracy, and therefore will provide another level of uncertainty in defining the particular situation which exists at any given location. Even the design and construction of the test phantom itself can play a part in defining statistical accuracy of performance assessment [Ramsdale (1989)]. Figure 3 taken from the latter reference indicates that not all phantoms provide the same low contrast-detail. The presence of detail in relation to the actual performance of a particular mammographic system fundamentally affects the results measured from phantom images, particularly when taking account of the wide variation in performance actually observed in practice [see Figure 4].

When attempting to build up a data base of test phantom images and the associated radiographic factors employed, then all possible operating conditions must be assessed in order to meaningfully combine data. When attempting to use this data to develop a predictive framework for relating changes in test phantom imaging performance to changes in radiographic factors, then the uncertainty on any predictive values must be assessed. This can only be undertaken by establishing the inter-relationship between the factors which can affect test phantom image quality, and which have been listed previously. Similarly, the frequency and magnitude of these effects needs to be assessed if a universally applicable expert system approach is to be developed.

The system which exists in the present problem is somewhat analogous to that which exists in control engineering. Fuzzy logic systems have been developed in order to handle input/output relationships which are not clearly defined and subject to statistical uncertainty. It would appear that concepts such as "Fuzzy Expert Systems" might equally apply to quality control in diagnostic radiology, when performance is being measured in terms of parameters capable of been affected by several inter-related technical factors and the inter-relationships themselves are not clearly defined.

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Law J. and Kirkpatrick A.E. (1988), Film Processing for Mammography, BJR Vol. 61 939-942.

Ibid. (1989), Films, Screens and Cassettes for Mammography, BJR Vol. 62 163-167.

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Ramsdale M.L. (1989), Image Quality and Test Phantoms in Mammography. In BIR Report 20, Optimisation of Image Quality and Patient Exposure in Diagnostic Radiology.

IV. Objectives for Next Period

1. To assess the results of the first two European trials of the Image Quality Criteria Document.

2. To continue to evaluate the factors which affect image quality measurements obtained from test phantoms in mammography with special consideration to the work presented at the Würzburg Workshop.

3. To develop a framework for incorporating test phantom measurements within the framework of the Image Quality Criteria.

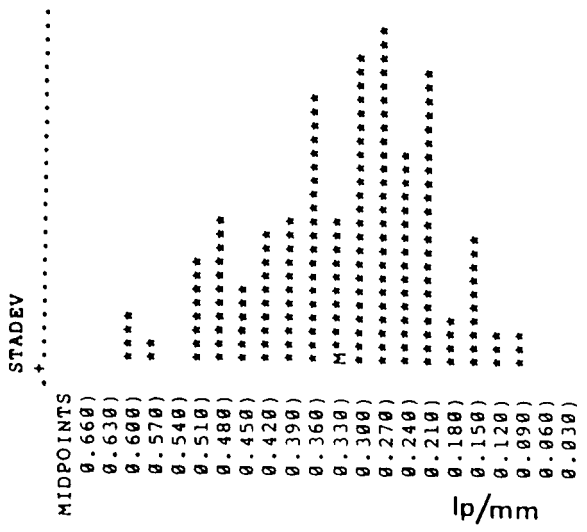

FIG. 1

Standard deviation of the test grid resolution, determined visually by 8 independent observers.

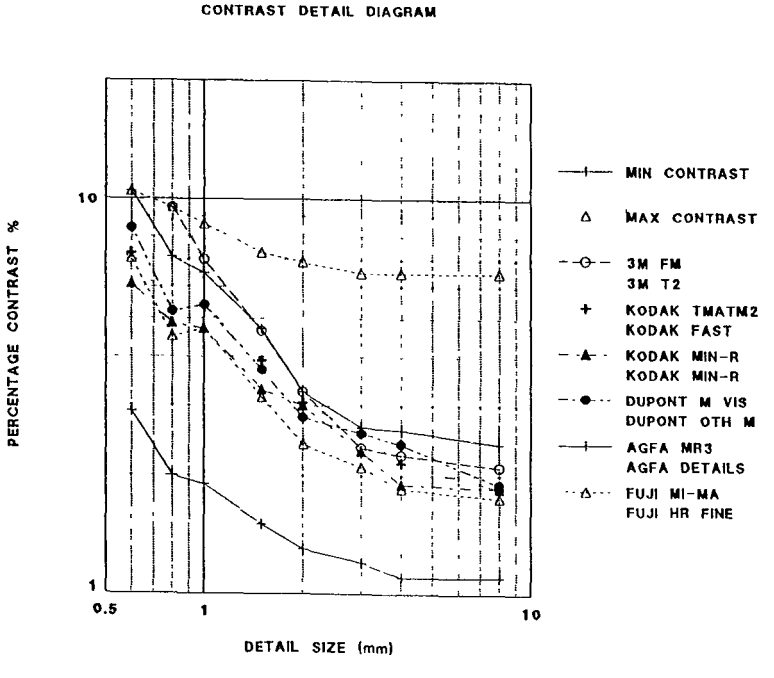

FIGURE 2

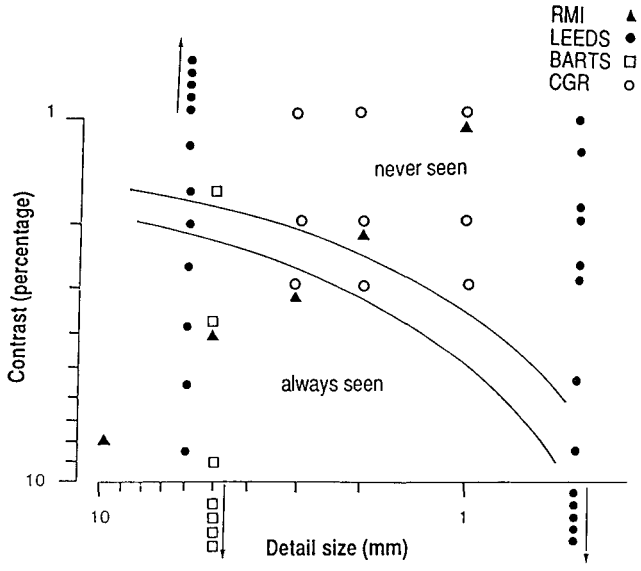

Figure 3 The availability of low-contrast detail in four image-quality test phantoms compared with the variation in low-contrast performance measured weekly on a particular mammographic system.

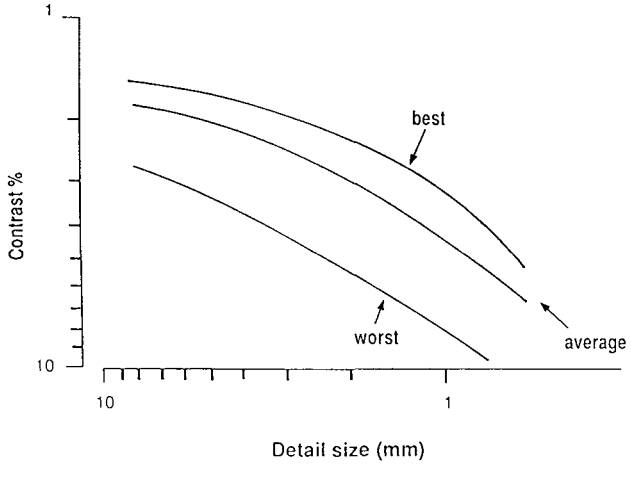

Figure 4 Low-contrast sensitivity plotted as minimum perceptible contrast against detail size for different mammographic systems showing worst, best and average performance.

Head Of Project 4: Dr D R Dance

II. Objectives for the reporting period

The principal objectives for the reporting period were (1) to continue with the preparation for publication of our work on grid optimisation using a computer model with a block phantom and (2) to make preliminary studies of the techniques necessary to include patient inhomogeneities within the model. These studies should include a survey of methodologies and a recommendation for the technique of choice, taking account of the use of a collision density estimator for the calculations. Close contact should be maintained with the University of Linköping to ensure a unified approach and to facilitate combination with their enhancement of our combined software which will include the transfer of contrast, resolution and noise by the receptor. Literature surveys of these effects should be made so that scientific input and guidance can be given to this aspect of the collaborative project.

III. Objectives for the next reporting period

The principal objectives for the next reporting period are (1) to develop and test a computer program incorporating patient inhomogeneities which can be used for the study and optimisation of anti-scatter grid design in diagnostic radiology, (2) to develop optimisation strategies for the use of the program, (3) to use the program to study the variation of the scatter-to-primary ratio in the image plane in the presence of inhomogeneities for a range of examination configurations, (4) to make preliminary studies of the application of the program in screen-film and digital radiology for both adult and paediatric examinations. Close contact should be maintained with the University of Linköping to ensure a unified approach and to facilitate combination with their enhancement of our combined software, which will include the transfer of contrast, resolution and noise by the receptor.

IV. Progress achieved including publications

Introduction and organisational details

The development of the computer program which models the use of anti-scatter grids in diagnostic radiology is being done jointly with the Department of Radiation Physics, University Hospital Linköping. The addition of patient inhomogeneities is being made in London and the improvement of the receptor model is being made in Linköping, but close contact is maintained throughout to ensure agreement in methodology at all stages. The majority of the programming and application work in London will be done by a PhD student who was appointed in March after the signed contract was received, and who will start work at the beginning of the academic year in October. In the meanwhile, important preparatory work has been done on (1) the methodologies which will be used in program development (2) understanding the factors which limit receptor performance and (3) the selection and purchase of computer hardware. In addition, significant time has been spent on preparing work done with our existing program for publication. This was felt to be of particular importance as very useful results were obtained in this work. A product can only have influence if it is widely disseminated and known.

To facilitate the collaboration between the two institutions, both project leaders have visited the other institution and close contact is made by fax and telephone. A combined visit has been made to the Finnish Centre for Radiation and Nuclear Safety (STUK) in Helsinki for discussions on both patient inhomogeneity and receptor modelling. A three month visit to work with Dr Tapiovaara in Helsinki is planned for a member of the Swedish team for the last quarter of 1993. The aim of the visit is to increase our expertise in image receptors and hence to improve the modelling in the program.

The present computer program and its applications

The computer program presently used to model the patient and the anti-scatter grid is based on the collision density estimator, which provides a means of calculating quantities of interest with high precision at points within the image plane. It has been used to calculate grid performance measures for a wide range of grid designs and imaging configurations, and has led to a number of publications. During the present period, papers on the advantages of low atomic materials for grid covers and grid interspaces, and on the performance of a range of commercially available grids have been revised and are now accepted for publication [1,2]. A paper has been prepared on the optimisation of grid design for situations with small, medium and large scattering volumes, and this is also accepted for publication [3]. A further paper has been prepared and submitted which sets out in detail the methodology used and the checks which have been made on the program [4]. An invited paper on grid selection has been given at the British Institute of Radiology meeting on *Image quality in the selection of equipment [5]* and two papers on the program and its applications have been accepted for the forthcoming CEC meeting on *Data analysis in quality control and radiation protection of the patient in diagnostic radiology and nuclear medicine* [6,7]. A paper has also been given at the *Svenska Läkaresällskapets Handligar* in Stockholm [8].

Addition of inhomogeneities to the program

Preliminary studies have been made of the methodologies available for incorporating patient inhomogeneities within the program. Three types of inhomogeneous phantom have been described in the literature. The first type describes the human body and the organs which it contains in terms of combinations of simple geometrical volumes defined in a closed form by the mathematical equations of their surfaces. Geometrical models are available for both children and adults and, for example, are used by the group at NRPB in Chilton. The second type of phantom describes the patient in terms of a series of voxels of known composition and size. Voxel data is available for children and is being developed for adults by the group at GSF in Neuherberg. The third type of phantom treats the patient as a series of slices, with the intersection of each organ with the slice being treated as a cylinder defined by a series of boundary points. Phantoms of this type are being used, for example, by the group at STUK in Helsinki. Contact has been made with the above three groups with a view to using the best available data for our purposes. It is clear from these contacts that phantom development for radiation dosimetry is still being actively pursued. In view of this there may be advantages in developing code which can use data obtained from any of the above models. It is therefore proposed to develop a voxel based model, but to use input data from any of the above sources (so that, for example, the geometrical model would be interpreted as its voxel based equivalent). This will retain maximum flexibility and will enable advantage to be taken of developments in models without major rewriting of the program. A further advantage is that this approach will be easier to incorporate into our existing model using the collision density estimator. A particular concern in developing the software is the

computation time likely to be required. High resolution is not necessary for our purposes and the voxel based approach will enable the resolution to be altered and the computation time to be decreased if necessary.

Addition of receptor transfer of noise, contrast and resolution to the program

A literature survey has been made of the factors which control and limit the performance of screen-film systems and of the barium fluoro-halide imaging plates used with many digital radiology systems. This has been summarised in [9,10]. It is clear that there is a qualitative understanding of the underlying physical principles which control the performance of these devices but that a phenomenological model may be needed for their effects to be incorporated into the program. Contact has been made with the CEC contracting group led by Prof. Malone in Dublin to explore how their measurements on image intensifier performance can be used for our work.

Conclusion

The objectives of the reporting period have been achieved. Both institutions feel that the collaboration has been symbiotic.

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Head of project 5: Dr. Padovani

II. Objectives for the reporting period

Modelisation of the problem solving process
Development of a conceptual model of Quality Control activities
Extension of the existent Knowledge Base
Introduction of log-book data in the inferential process

III. Progress achieved including publications

The development of our Knowledge-Based System (KBS) has followed a general methodology. An abstract and formal description (conceptual model) of expertise in Quality Control (QC) was the first phase of the developmental process of the KBS. It was of interest to individuate facts, physical objects, concepts of the domain, as well as relations among them. A common technique was used in which objects are reported as nodes and relations as arcs connecting nodes (semantic network). In QC every single procedure consists in checking the efficiency of a component (sub-system) of the radiological equipment. To this purpose a test tool is used and the results of the procedure (the measurements of one or more observables) must be compared with reference values or standards. An overview of the objects involved and their relational links is shown in figure 1 as a semantic net.

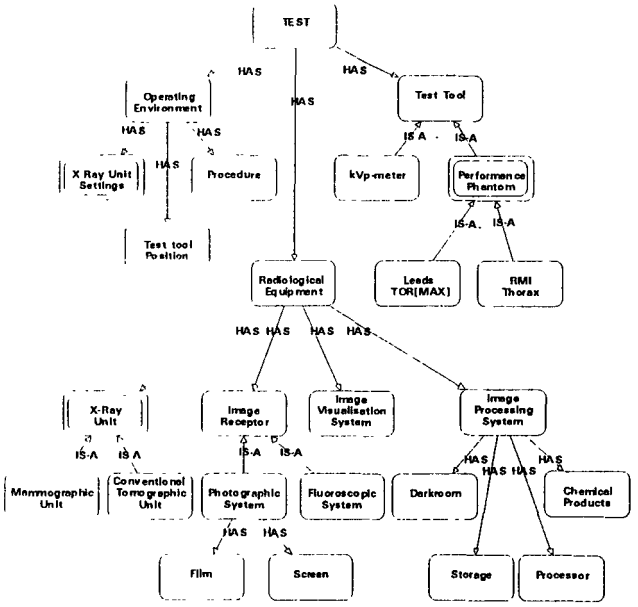

Figure 1. Global object representation in the conceptual model of a QC test. Has and is-a links are used to identify structural and conceptual hierarchies, respectively. Double-encircled boxes will be further expanded in the forthcoming figures.

This representation is centred on the object TEST, whose properties include the characteristics of the radiological equipment to be checked, the test-tool used and the operating environment under which the test has been executed. Every node of the network can be further expanded to show more objects and other relational links (figure 2).

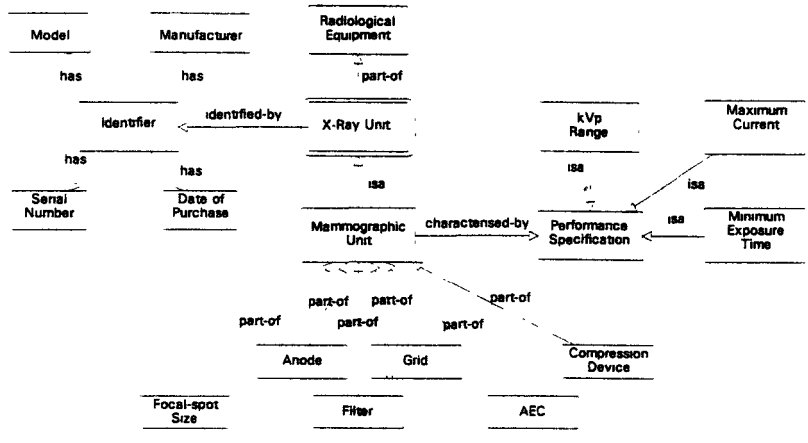

Figure 2. A mammographic unit is represented as a specific X-ray unit with structural/functional characteristics.

As our first implementation of KBS addresses the task of QC for mammographic systems, a special concern was due to the performance phantom. In figure 3 one of them, the Leeds TOR[MAX] phantom is represented with its main properties. Each property of the phantom is called a finding and is characterised by a list of attributes. This part of the conceptual model has been reported in the previous progress report and elsewhere (1).

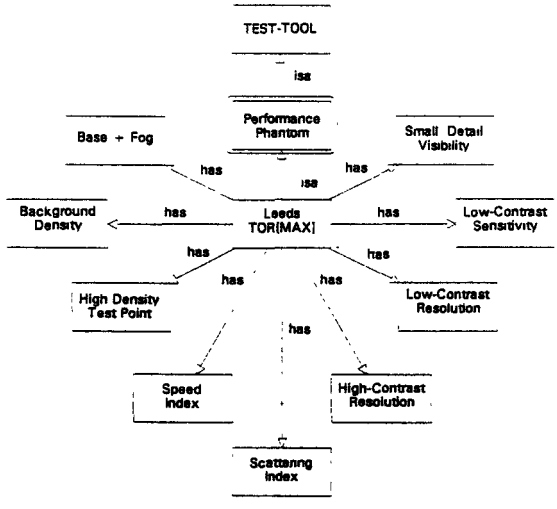

Figure 3. The mammographic phantom Leeds TOR[MAX] is described in terms of its physical observables.

Similarly, an analysis has been carried out on the dynamics of object manipulations. QC tasks have been structured into three levels of abstraction associated to *plan*, *phase* and *generic action* activities. Planning is the ability of humans to select the most appropriate set of actions to achieve a specified goal. For example, plans are aimed at:

- verifying the equipment conformity with purchase specifications at acceptance testing;
- optimising the equipment performance;
- executing routine tests;
- consulting the log-book.

Every plan includes high-level tasks, here called *phases*. In the plan reported in figure 4, phases are chosen so as to maintain a wide general scope, independent of the type of test under consideration. Each phase is formed by sub-tasks of finer granularity, the generic actions. The relations between generic actions represent the logical connections or the chronological sequences of the parts of each QC procedure Figure 5 shows the flow of generic actions performed during a diagnostic process.

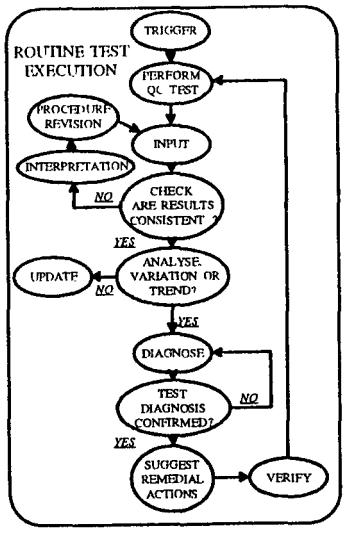

Figure 4. A plan for executing routine tests (topmost level description of a QC task).

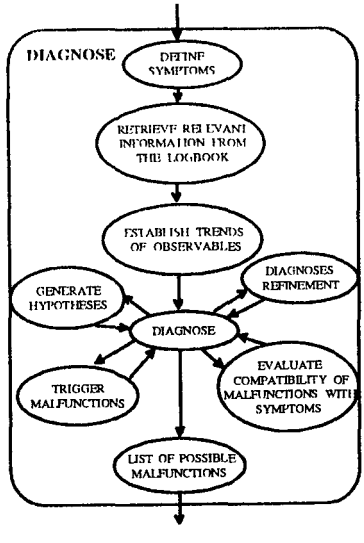

Figure 5. The diagnostic phase of the plan Routine Test Execution is expanded at the generic action level.

The diagnostic system acquires greater power by accessing the information stored in the log-book (database) to allow comparisons, discover trends, detect abnormal functions even before control limits are reached. Among the features of the log-bbok actually implementd in the knowledge-base are included: protocols of QC programmes, general reference standards, departmental standards, baselines, tolerances, limiting values, results of test procedures, histories of previously debugged problems. The structure of the log-book model allows every user to configure his/her own log-book according to the QC programme protocol actually implemented in his/her department. Every software tool for database management can be interfaced to the KBS by simply adding an appropriate module, thus making possible exchange of information from an external log-book to the knowledge base.

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Head of project 6: Prof. Vañó Carruana

IIa. Objectives for the reporting period

* Application of image quality techniques by using different test objects and phantoms.

* Patient dose measurements and evaluation of the obtained reductions in optimization processes.

* Obtention of experimental data to contribute to the elaboration of the expert system in diagnostic radiology quality control.

IIb. Objectives for the next reporting period

* Application of the anatomical image criteria proposed by the EC expert group and comparison with the obtained results using test objects.

* Study of the obtained doses and image quality with several combinations of intensifying screens-films according to different types of radiological examinations.

* Analysis of the optimization processes and results of a second evaluation on image quality and doses in mammography, carried out in the Madrid area.

III. Progress achieved including publications

Requirements that should meet the test objects and phantoms used to evaluate image quality in conventional diagnostic radiology have been analyzed. The following conclusions have been found:
1) They should be easily employed both on the table and on a wall stand; 2) The beam attenuation and scattering properties of the human tissue should be simulated in order to ensure that entrance doses be similar to those of a standard patient; 3) The points where the background optical density must be measured should be clearly identified; 4) Simple test pattern of line pairs (both for high and low contrasts) which could be arranged at an angle of less than 45° to the strips of the grid and allow detection values between 0.5 to 5.0 lp/mm should be included; 5) They should also contain copper meshes to evaluate appropriate film-screen contact areas; 6) Some marks should be present in order to check the alignment of the light field edges with the X-ray field; 7) Some details with different sizes and absorptions, comparable to those normally found in patient radiographs, enabling the detection of changes in the actual kV should be included; 8) A calibrated step wedge should be included; 9) They should contain a lead strip that allows a further sensitometric exposure on the same film; 9) The test images viewing conditions should be clearly defined; 10) Criteria for scoring the obtained image should be attached indicating the expected and recommended values for certain techniques and film-screen combinations and, 11) The possibility of designing mobile objects, in order to avoid the capacity of the observer to learn and remember their position,

would be eventually valuable.

Using the TOR(CDR) test by the Leeds University in different placements between appropriate attenuators (similar to the ANSI basic phantom), a new test object that allows the simulation of real examinations (those contained in the CEC, XII/173/90 document on Image Quality Criteria in Diagnostic Radiology) has been designed. The results obtained with this phantom have been used and compared with those of TOR(CDR) and those using Wellhofer test (designed according to the DIN standard), in more than 12 X-ray rooms.

The sensitivity of the new designed phantom has been also analyzed, both for spatial resolution and for low and high contrast thresholds, and for different kV and optical densities.

In mammography, the image quality controls have been carried out using the TOR(MAX) phantom by the Leeds University, for different screen-film combinations. Evaluations have been done on 21 units of the Madrid area, measuring also entrance air kerma and estimating the average glandular dose of a sample of patients. A comparison of these dose values with the obtained image qualities and the equipment conditions has been made. It has been analyzed by groups: mamographic units of hospitals, outpatient centres, private centres and those dedicated specifically to screening programmes. Measured entrance air kerma values have ranged from 2.9 mGy (in outpatient centres) to 6.4 mGy (in screening units), while the obtained image qualities, assigning a global score using the perceived test details, vary from 33 (in outpatient centres) to 42 (in screening units). The performed quality controls have provide recommendations to reduce doses.

It has been carried out estimations of patient dose reductions as a consequence of a pilot quality control programme, both in paediatrics and in adults, in thorax radiology rooms, achieving entrance dose reductions up to 60% by correcting some anomalies due to the generator. In traumatology rooms, values of lumbar spine and hip and pelvis doses were reduced up to 35% by adding filtration and adjusting radiographic techniques.

One of the most important achieved optimization has been carried out in a centre, which participated in the 1991 CEC trial and in which, in spite of being X-ray equipment and processors in excellent order, lumbar spine entrance patient doses were the highest of the european trial. The optimization, in this case, consisted in modifying the techniques applied by radiographers (basically increasing kV and distance) and using one darkness step below the normal, in the automatic exposure. Thus, dose values were reduced under the reference ones.

In paediatrics the optimization has been applied to chest, abdomen and mictional cystoureterography. The use of high speed systems (800, when it is possible due to the image characteristics to be viewed), to withdraw the bucky, strict collimation, interrupted use of fluoroscopy and correct use of the automatic exposure (selecting appropriately the central or lateral sensors and darkness factors), have allowed dose reductions up to a factor 3.

The evolution of annual effective equivalent doses in different examinations has been analyzed for one of the hospitals participating in the quality control pilot programme since 1987. In this way, a global indicator of the achieved benefit after the application of the

mentioned programme has been obtained.

Finally, our group has offered a wide experimental measurement database in mammography using the Leeds TOR(MAX) object, to contribute to the knowledge base of the expert system on quality control, which is being developed in Udine. A number of images obtained in different conditions (kV, optical density, etc.) have been performed and analyzed in order to simulate possible anomalies of mammographic equipment. It has been necessary to carry out a previous study of the sensitivity of the Leeds phantom to detect anomalous working of mammographic equipment.

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Progress Report

Contract: FI3P-CT920052 **Sector:** C22

Title: Patient dose from radiopharmaceuticals.

1) Smith. MRC
3) Petoussi GSF
4) Evans. Univ. London

I. Summary of Project Global Objectives and Achievements

The general objective of this project is to provide accurate information on radiation absorbed doses to patients from radiopharmaceuticals. In particular, increased effort is required to accumulate reliable paediatric dosimetry data, and to improve the physical basis of dose calculation. This objective is being achieved by:

1. Accumulating biokinetic data for some new radiopharmaceuticals such as Tc-99m-labelled heart and renal agents, In-111-labelled antibodies etc, by serial biodistribution studies in patients and healthy volunteers. Alternatively, dosimetry of the important group of short-lived PET tracers is being investigated by means of modelling (e.g. O-15 water).
2. Giving special attention to the biokinetics and dosimetry for children and new-born infants (with Tc99m DMSA as first priority), with assessment of the diagnostic quality of imaging.
3. Extending the principle of "voxel" phantoms to internal dosimetry. This concept, previously developed for use with external irradiation, is based on CT-derived anatomical data and Monte Carlo simulation of photon transport and presents the opportunity to determine accurately unique S-values for radiopharmaceutical dose estimation in individual persons.

The results have been used to quantify organ doses as well as effective dose equivalents and effective doses for some important new radiopharmaceuticals. Considerable differences in biokinetics have been demonstrated between adults, children and new-born. Paediatric studies on an outpatient basis have been found to be unsatisfactory, and therefore the recent inclusion of the ICH (University of London) in the project has proved to be a considerable advantage and resulted in a highly productive study on children attending as in-patients for Tc-99m DMSA renal scanning. This work has drawn attention to the limitations of the small series of anthropomorphic phantoms (MIRD) for dose calculations covering a wide range of children's age and size, and emphasizes the importance of the work being carried out at GSF on CT-based phantoms for individual subjects. Present results on a 7 year old child indicate some significant differences in SAF values compared with those based on MIRD phantoms. The extent to which such differences influence overall organ and effective dose estimates requires further exploration.

In summary, the results of this project enable comparison of different radionuclide labels and of different pharmaceuticals, in view of the need to balance diagnostic benefit against radiation dose and to determine the appropriate level of administered activity in order to avoid unnecessary radiation exposure of the patient.

Head of project 1: Dr. Smith.

II. Objectives for the reporting period

1. Biodistribution and dosimetry of In-111 Antimyosin have been investigated in 12 subjects with inflammatory myopathies. Comparative studies on 2 subjects with no evidence of disease have recently been completed.
2. Accurate measurements (by attenuation-corrected conjugate counting, SPECT) of myocardial and other organ uptake 1-2h after administration of Tc-99m myocardial perfusion imaging agents are in progress, with particular reference to Tc-99m P53 (tetrofosmin).
3. A predictive model, based on the continuous measurement of arterial blood concentration has been developed to estimate the dosimetry of infused O-15 water. Provisional results have been reported.
4. Paediatric dosimetry of Tc-99m DMSA has been attempted on an outpatient basis, with only limited success.

Next reporting period

1. In-111 Antimyosin biodistribution data on 2 control subjects will be compared with results from 12 subjects with myopathies. All data and dosimetry from these studies will be prepared for publication.
2. Myocardial and other organ uptake will be accurately measured in subjects given Tc-99m P53 (tetrofosmin) both at stress and rest, and the results compared with uptakes already obtained in 12 subjects given Tc-99m MIBI.
3. Some data used in the O-15 water model are being revised for reassessment of organ and effective doses and comparison with current models.
4. Paediatric Tc-99m DMSA results will be assessed for their value as supplementary information.

III. Progress achieved including publications

1. Biodistribution and radiation dosimetry of In-111 Antimyosin antibody

Serial biodistribution studies have been completed in 12 patients (5M, 7F) with inflammatory myopathies who were referred for immunoscintigraphy with In-111-Antimyosin antibody (A-M). The aims of the study were to investigate the extent of myocardial and skeletal muscle involvement in this disease but the course of the investigations provided the essential data for radiation dose estimation, and the present report is therefore confined to that aspect of the overall study.

Methods

Following injection of 2mCi (74 MBq) of In-111 A-M, patients were monitored by whole-body scanning using an IGE 400AT gamma-camera fitted with medium energy collimator. Scans were made at 5 min, 30 min (in only 5 patients), 2, 7, 24, 48 and 72h post-injection. Overlapping, longitudinal split-field scans were obtained with the patient both supine and prone to allow the determination of conjugate organ counts. Complete collections of urine were made between 0 and 72 hours in the intervals 0-2, 2-4, 4-7, 7-24, 24-48 and 48-72h and the total activity in each time interval was measured using a calibrated bulk-sample detector. No faecal collections were necessary since there was no evidence of activity in the GI tract

Table 1 In-111 Antimyosin antibody studies - organ uptake values (maximum) and radiation dose estimates

Organ	No background correction		Full background correction		Thigh background correction	
	uptake (%)	dose ($mGy.MBq^{-1}$)	uptake (%)	dose ($mGy.MBq^{-1}$)	uptake (%)	dose ($mGy.MBq^{-1}$)
Ovaries		$8.07.10^{-2}$		$1.0.10^{-1}$		$9.03.10^{-2}$
Testes		$3.74.10^{-2}$		$5.48.10^{-2}$		$4.58.10^{-2}$
Red marrow *		$3.0.10^{-1}$	12.6	$3.01.10^{-1}$		$3.01.10^{-1}$
Colon		$7.41.10^{-2}$		$9.40.10^{-2}$		$8.38.10^{-2}$
Lungs *	9.2	$2.24.10^{-1}$	5.0	$1.31.10^{-1}$	6.8	$1.71.10^{-1}$
Stomach		$1.15.10^{-1}$		$1.09.10^{-1}$		$1.12.10^{-1}$
Bladder *		$1.37.10^{-1}$		$1.46.10^{-1}$		$1.41.10^{-1}$
Breast		$5.99.10^{-2}$		$5.80.10^{-2}$		$5.88.10^{-2}$
Liver *	15.7	$5.70.10^{-1}$	12.1	$4.33.10^{-1}$	14.1	$5.03.10^{-1}$
Oesophagus		$9.21.^{-2}$		$8.27.10^{-2}$		$8.83.10^{-2}$
Thyroid		$4.87.10^{-2}$		$6.45.10^{-2}$		$5.62.10^{-2}$
Skin		$3.90.10^{-2}$		$4.63.10^{-2}$		$4.26.10^{-2}$
Bone surface		$1.62.10^{-1}$		$1.75.10^{-1}$		$1.69.10^{-1}$
Kidneys *	9.7	1.08	6.9	$7.76.10^{-1}$	8.5	$9.56.10^{-1}$
Spleen *	3.5	$6.31.10^{-1}$	2.2	$3.87.10^{-1}$	2.7	$5.15.10^{-1}$
Heart *	10.2	$2.86.10^{-1}$	5.8	$1.60.10^{-1}$	9.1	$2.33.10^{-1}$
ED ($mSv.MBq^{-1}$)		$1.76.10^{-1}$		$1.56.10^{-1}$		$1.66.10^{-1}$
ED ($mSv.74MBq^{-1}$)		13.02		11.54		12.28

* Source organ

throughout these studies. Blood samples were withdrawn at the same times as the scans and aliquots of whole-blood and plasma were counted against diluted standards of the administered dose in an LKB Compugamma counter. Whole-body anterior and posterior images were analysed by regions-of-interest (ROI) to give estimates of serial total-body and source organ geometric mean counts. Source organs were heart, lungs, spleen, liver, kidneys, bladder and bone-marrow. Organ ROIs were corrected for blood background, radioactive decay and tissue attenuation and then referred to phantom calibration data to establish absolute activity content of the organs. Attenuation correction was based on a transmission scanning technique. Residence times for whole-body (less bladder activity) and source organs were obtained by mathematical description of retention data and used with a dose-calculation program MIRDOSE 2 (Oak Ridge Associated Universities) to give dose estimates to 25 target organs, from which the Effective Dose (ED) was calculated.

Results

Whole-blood activity fell from 21.3% dose.l^{-1} at 30 min to 5.6, 3.1 and 2.0 % dose.l^{-1} at 24, 48 and 72h, and was essentially confined to plasma. Whole-body biological retention fell to 64% of dose at 72h, in good agreement with urinary excretion data, and showed a final retention component with a 8.5 day half-time. Highest organ uptake was observed in liver (14%), but kidneys (8.5%) received the highest dose. Blood background corrections of organ uptake were often large and difficult to apply because of adjacent organs with high activity, and Table 1 shows the influence of such corrections compared with uncorrected values. For this reason, the use of a blood background correction obtained from thigh activity may be a suitable compromise. The EDs for the three methods, however, are quite similar, about $1.66.10^{-1}$ mSv. MBq^{-1}, predicting an ED of 12.3 mSv for a 74 MBq injection.

2. Dosimetry of Tc-99m-labelled myocardial perfusion imaging agents

Results of dosimetry studies on a newly developed radiopharmaceutical Tc-99m P53, 1,2-bis[bis(2-ethoxyethyl)phosphino]ethane (tetrofosmin), have been reported previously and a further article on this subject has been published (Higley *et al*, J. Nucl. Med, 1993). At this centre there is continued clinical interest in this substance and in its comparison with similar agents in current use. A study has been initiated to determine accurately the uptake into the myocardium and other organs, such as liver, kidneys, spleen and lungs, 1 to 2 hours post-injection. Twelve studies have now been completed on patients given Tc-99m MIBI both at rest and under stress and studies on a similar group of patients given Tc-99m P53 have recently begun.

Methods

Patients undergo SPECT and static imaging (AP, PA and L lateral) of the chest, 1 to 2h following injection of 500 MBq Tc-99m myocardial perfusion imaging agent with the heart under dobutamine stress. Four days later, they have a PA transmission scan using a Tc-99m flood phantom prior to a second injection of Tc-99m agent at rest. The SPECT and static imaging are repeated 1 to 2h after this injection. The effective body thickness (cm of water) at a given organ site is determined from the transmission scan, and the organ count rate per MBq uptake is deduced from this information and a calibrated relationship between Tc-99m source count rate and water thickness. Thus the organ uptake can be determined as percentage of

administered dose with accurate correction for tissue attenuation.

Results

This work is ongoing and few results are available so far. However, provisional analysis of the Tc-99m MIBI data show that the mean myocardial uptake (n=12) was 1.59 (± 0.43)% of dose at stress and 1.50 (± 0.38)% of dose at rest. The small increase in uptake at stress (0.09 ± 0.15% of dose) was not significant.

3. PET dosimetry studies - A kinetic model for the radiation dosimetry of intravenously administered O-15 water. (In collaboration with the MRC Cyclotron Unit, Hammersmith Hospital)

The short physical half-life (2.04 min) of O-15 complicates dosimetry by conventional imaging, and predictive models based on organ blood flow may offer a better solution. The aims of the present work were:-

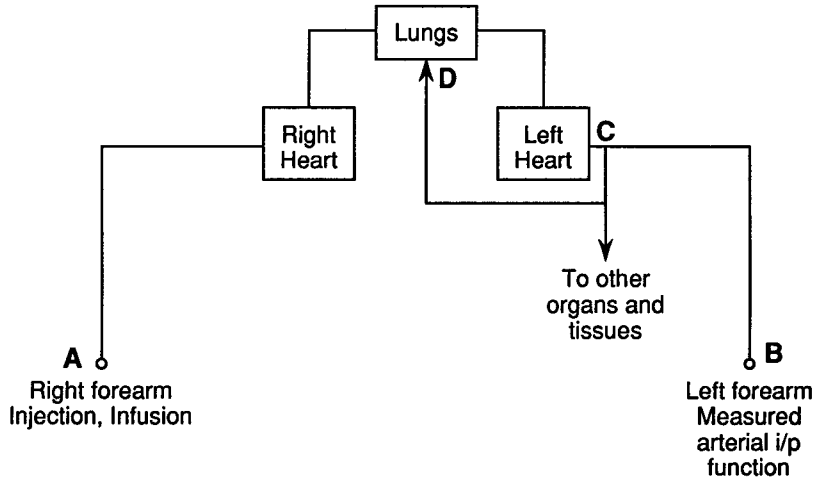
1. To develop a model to predict residence times of O-15 water in organs based on the measurement of the time course of arterial blood concentration.
2. To use the model to derive organ doses and the Effective Dose (ED).
3. To compare the dose estimates with those predicted by other models.

Methods

The model is shown diagrammatically in Fig. 1. Both the O-15 water activity infused at A and the resulting arterial blood concentration at B are continuously monitored. The measured concentration at B, decay corrected to C (left heart), represents the arterial input function to organs. The time-course of O-15 water concentration (C_t) in any organ can be obtained by convolution of its arterial input function with its transit time function, which are determined from the organ blood flow and water distribution space (Fig. 1). These values are obtained from the literature. The predicted values of C_t were integrated to yield organ residence times which were used with the MIRDOSE 2 dose-calculation program to give dose estimates to 25 target organs from which the ED was calculated. Special calculations were necessary to estimate residence time in heart, lungs and liver (portal flow).

Results

Our preliminary results were presented at the 1993 meeting of the British Nuclear Medicine Society. They indicated that the ED predicted by the above model was 9.0 (± 1.3) x 10^{-4} mSv.MBq^{-1} (n=5). This result was significantly higher than that predicted by current models based on uniform distribution of O-15 water (4.5 x 10^{-4} mSv.MBq^{-1}) but considerably less than that given by a microsphere-type model (3.0 x 10^{-3} mSv.MBq^{-1}) where no recirculation of O-15 water from organs is assumed. However, on further reconsideration of our data prior to publication, a discrepancy was discovered in the administered activity values, resulting from a calibration error. This error is being investigated prior to reassessment of the dosimetry, and the revision is expected to lead to a slightly higher dose estimate than formerly predicted.



Organ concentration (C_t $\mu Ci.g^{-1}$):–

$$C_t = F.f(BC).Ca * \exp\left[-\left(\frac{F}{Vd} + \lambda\right)t\right]$$

* denotes convolution

Ca : arterial i/p function ($\mu Ci.ml^{-1}$)

F : organ blood flow ($ml.g^{-1}.min^{-1}$)

Vd : relative water distribution space

λ : $^{15}0$ radioactive decay constant (0.34 min^{-1})

f(BC) : decay correction for delay B–C

4. Paediatric dosimetry of Tc-99m DMSA

Altogether, 21 attempts have been made to study biodistribution and dosimetry in paediatric subjects (9 months to 9 y) attending out-patient clinic for diagnostic renal DMSA scintigraphy. However, in only three of these studies was it possible to obtain sufficient measurements to establish the full kidney uptake and retention patterns. In view of the recent inclusion of the Institute of Child Health (University of London) in this CEC contract, and their ability to perform complete DMSA biodistribution studies on paediatric in-patients, further attempts on out-patients at this Institute will be discontinued. The information so far accumulated will be assessed for its value in supplementing the results from the ICH. For example, in one 4 year old girl on whom four measurements were made on the day of injection and one at 24h, kidney uptake rose to a steady value of 34% after about 5h and total body activity fell to 75% of dose at 24h. The estimated Effective Dose was 2×10^{-2} $mSv.MBq^{-1}$. Uptake of $^{99}Tc^{m}$ in knees and other joints was observed in most patients and in the knees amounted to about 3 to 5% of the administered activity.

5. Publications

Smith, T., Tong, C., Lammertsma, A., Butler, K.R., Schnorr, L., Watson, J., Ramsay, S., Clark, J.C. and Jones, T. (1993). A kinetic model for the radiation dosimetry of intravenously administered $H_2{}^{15}O$. Nucl. Med. Comm., 14, 300.

Higley, B., Smith, F.W., Smith, T., Gemmell, H.G., Das Gupta, P., Gvozdanovic, D.V., Graham, D., Hinge, D., Davidson, J. and Lahiri, A. (1993). Technetium-99m -1,2-bis[bis(2-Ethyoxyethyl) Phosphino]Ethane: Human biodistribution, dosimetry and safety of a new myocardial perfusion imaging agent. J. Nucl. Med., 34, 30-38.

Mattsson, S., Johansson, L., Nosslin, B., Smith, T. and Taylor, D.M. (1993). Radiation dose to patients from radiopharmaceuticals. ICRP Publication 62 (Addendum 1 to ICRP Publication 53), Pergamon Press, Oxford.

Head of project 3: Dr. Petoussi

II. Objectives for the reporting period

The main objectives of this reporting period were to obtain the specific absorbed fractions (SAF) and specific absorbed energies (SEE) in various "target" organs of the body, from sources of monoenergetic photons in various "source" organs for a seven-year old child. The child was modelled by a voxel phantom which was constructed from computed tomographic data of a real child (1). The transport of photons in the body was simulated by a computer program based on Monte Carlo methods.

Similar studies have been performed until now with the help of MIRD anthropomorphic phantoms (adult and paediatric) (2,3) which are mathematical phantoms describing the geometry of the body and its organs with simple equations. One of the main objectives of this study, was, besides obtaining dose data for a child, to investigate to what extent the model, MIRD or voxel, influences the resulting dose equivalents. Also, the degree of improved accuracy of the current data should be examined, in view of doses delivered to individual nuclear medicine patients.

The objective of the next reporting period is, using the SAF values, to calculate the photon component of the dose-equivalent rate in a given target organ from a given radionuclide that is a present in a source organ. This will be achieved by incorporating biokinetic data and the nuclear transformations of the nuclides. Software has to be developed for this purpose in order to be able to calculate the doses from several radiopharmaceuticals, old and new. Another objective is to investigate the influence of patient size on the dose; this will be done by changing the size of the voxels and thus of the phantoms. Finally, preparation for publication of the obtained data is under way.

III. Progress achieved including publications

Introduction

Before beginning this project, the voxel phantoms, which are still under development at the GSF, have been used for simulation of external exposures only. Integration of internal emitters into the computer program provided an extension of their application. In the previous contract period, calculations of SAF for an 8 week old baby took place. The progress achieved in this reporting period involves the estimation of SAF for a seven year old child.

Method and Results

The phantom of the child was constructed from computed tomographic data obtained with a very fine CT of a real patient. In this way, a greater degree of realism can be achieved with a voxel-type phantom than with a mathematical MIRD type phantom concerning the shape and location of the organs and the derivation of red bone marrow; the latter is assessed in each single skeletal voxel directly from the CT data. The child had a weight of 21.7 kg and a height of 1.15 m; seven different media were simulated according to their elemental composition: bone, bone marrow, soft tissue, skin, lung tissue, muscle

tissue and air.

The monoenergetic photons simulated were in the energy range 0.02-3.00 MeV (see Table 1 for a detailed list of energies). The Monte Carlo calculations were performed on an intel ipsc/2 hypercube parallel computer, achieving a statistical accuracy which lies generally below 5 %.

SAF were obtained for 24 source organs and over 100 target organs. As an example of the data obtained, Table 1 gives the SAF values in g^{-1} for thyroid as the source organ and for the most important target tissues.

The SAF values obtained were used to calculate the photon component of the dose-equivalent rate in a given target organ for some radionuclides deposited in a given source organ. For electrons, complete absorption in the source organ was assumed. Data on the biokinetic behaviour of the incorporated activities was taken from ICRP 53 (2). Table 2 gives the dose equivalents per unit activity incorporated for $^{99}Tc^{m}$, ^{131}I and ^{123}I. For the sake of comparison, the dose equivalents of the MIRD phantoms of 5 and 10 year olds are shown together with the dose equivalents for the 7 year old child. The data for the MIRD phantoms were taken from Cristy and Eckerman (2).

Comparison of data obtained with the voxel and MIRD-type phantoms

Table 3 gives the calculated SAF values for the 7 year old child (21.7 kg), together with the SAF of a 5 year old child (19 kg) and a 10 year old child (32 kg) from the data of Cristy and Eckerman. It can be seen, that different modelling does result in different organ doses, therefore it would be justified to use the voxel phantoms instead of the mathematical phantoms; the differences in SAF, however, become smooth in the dose values depending on which organs the radionuclides are concentrated (Table 2).

However, more data are needed to decide whether a more detailed modelling of the human body brings advantages to individual patient dosimetry and for which radiopharmaceuticals and tissues. For that purpose, it is absolutely necessary to develop suitable software to include biokinetic data and nuclear tranformations with SAF values and perform calculations of dose equivalents per unit activity incorporated. Furthermore, additional calculations with more phantoms will be necessary.

References

1. Zankl, M., Veit, R., Williams, G., Schneider, K., Fendel, H., Petoussi, N., Drexler, G. 1988. The construction of computer tomographic phantoms and their application in radiology and radiation protection. Radiat Environ Biophys **27**:153-164

2. Cristy, M. and Eckerman, K.F. 1987. Specific absorbed fractions of energy at various ages from internal photon sources. Oak Ridge National Laboratory Rep. ORNL/TM-8381:Vol. **1 -7**

3. International Commission on Radiological Protection. 1987. ICRP Publication 53. Radiation dose to patients from radiopharmaceuticals. Pergamon Press.

Table 1: Specific absorbed fraction (SAF) of photon energy in 1/g, for a 7 year old child (21.7 kg)

Source Organ : THYROID

Energy [MeV]

Target Organ	0.020	0.030	0.040	0.050	0.060	0.070	0.080	0.090	0.100	0.150
ADRENALS	0.4705E-08	0.4120E-06	0.1622E-05	0.2505E-05	0.2708E-05	0.2912E-05	0.2940E-05	0.2894E-05	0.2926E-05	0.2687E-05
BLADDER WALL	0.0000E+00	0.1443E-10	0.4902E-08	0.2110E-07	0.3728E-07	0.4728E-07	0.6859E-07	0.6475E-07	0.5869E-07	0.9281E-07
BRAIN	0.1424E-06	0.1231E-05	0.2693E-05	0.3550E-05	0.3881E-05	0.3982E-05	0.4024E-05	0.4028E-05	0.4029E-05	0.4125E-05
EYES	0.1774E-08	0.5845E-06	0.2323E-05	0.3579E-05	0.4212E-05	0.4513E-05	0.4630E-05	0.4665E-05	0.4746E-05	0.5021E-05
HEART	0.1149E-05	0.1341E-04	0.1967E-04	0.1951E-04	0.1800E-04	0.1646E-04	0.1531E-04	0.1451E-04	0.1397E-04	0.1278E-04
LARGE INTESTINE WALL	0.0000E+00	0.3575E-07	0.2162E-06	0.4296E-06	0.5518E-06	0.6279E-06	0.6643E-06	0.6925E-06	0.6809E-06	0.7217E-06
SMALL INTESTINE WALL+C.	0.9695E-11	0.1771E-07	0.1299E-06	0.2698E-06	0.3466E-06	0.3969E-06	0.4260E-06	0.4424E-06	0.4512E-06	0.4794E-06
KIDNEYS	0.7803E-10	0.9525E-07	0.4349E-06	0.7512E-06	0.9376E-06	0.1035E-05	0.1066E-05	0.1092E-05	0.1105E-05	0.1122E-05
LIVER	0.4262E-07	0.1225E-05	0.2876E-05	0.3577E-05	0.3749E-05	0.3722E-05	0.3649E-05	0.3557E-05	0.3466E-05	0.3295E-05
LUNGS	0.1552E-04	0.3842E-04	0.3672E-04	0.3092E-04	0.2649E-04	0.2357E-04	0.2170E-04	0.2055E-04	0.1969E-04	0.1846E-04
OVARIES	0.0000E+00	0.3745E-08	0.1763E-07	0.1130E-06	0.1380E-06	0.2026E-06	0.2054E-06	0.2430E-06	0.3082E-06	0.3112E-06
PANCREAS	0.1222E-09	0.2554E-06	0.1025E-05	0.1739E-05	0.1955E-05	0.2107E-05	0.2095E-05	0.2173E-05	0.2148E-05	0.2031E-05
SKIN	0.1480E-04	0.9958E-05	0.6806E-05	0.5204E-05	0.4412E-05	0.4036E-05	0.3810E-05	0.3717E-05	0.3665E-05	0.3768E-05
SPINAL CORD	0.4054E-05	0.1859E-04	0.2173E-04	0.2041E-04	0.1841E-04	0.1694E-04	0.1601E-04	0.1536E-04	0.1498E-04	0.1425E-04
SPLEEN	0.3207E-07	0.8268E-06	0.1907E-05	0.2499E-05	0.2651E-05	0.2659E-05	0.2651E-05	0.2563E-05	0.2556E-05	0.2460E-05
STOMACH WALL	0.7449E-09	0.2728E-06	0.1038E-05	0.1608E-05	0.1849E-05	0.1917E-05	0.1934E-05	0.1929E-05	0.1876E-05	0.1887E-05
THYMUS	0.6101E-03	0.5068E-03	0.3325E-03	0.2328E-03	0.1806E-03	0.1543E-03	0.1382E-03	0.1302E-03	0.1250E-03	0.1208E-03
THYROID	0.3059E-01	0.1140E-01	0.5510E-02	0.3397E-02	0.2528E-02	0.2179E-02	0.1966E-02	0.1894E-02	0.1861E-02	0.1941E-02
TISSUE	0.1792E-04	0.1528E-04	0.1146E-04	0.9033E-05	0.7636E-05	0.6901E-05	0.6433E-05	0.6193E-05	0.6055E-05	0.5980E-05
UTERUS	0.0000E+00	0.3006E-09	0.1960E-07	0.5529E-07	0.7385E-07	0.9007E-07	0.1282E-06	0.1373E-06	0.1428E-06	0.1707E-06
SKELETON	0.1374E-04	0.4370E-04	0.5021E-04	0.4429E-04	0.3637E-04	0.2977E-04	0.2454E-04	0.2075E-04	0.1783E-04	0.1103E-04
RED BONE MARROW	0.2298E-05	0.5816E-05	0.6389E-05	0.6119E-05	0.5906E-05	0.5784E-05	0.5729E-05	0.5744E-05	0.5784E-05	0.6065E-05
TOTAL BODY	0.2194E-04	0.1879E-04	0.1538E-04	0.1255E-04	0.1056E-04	0.9283E-05	0.8372E-05	0.7807E-05	0.7412E-05	0.6732E-05
STOMACH CONTENTS	0.2285E-09	0.2404E-06	0.9612E-06	0.1461E-05	0.1714E-05	0.1790E-05	0.1818E-05	0.1821E-05	0.1824E-05	0.1832E-05
TESTES	0.0000E+00	0.0000E+00	0.0000E+00	0.5740E-08	0.6785E-08	0.8337E-08	0.2290E-07	0.8385E-08	0.8086E-08	0.2378E-07

Table 1 (continued)

Target - Organ	0.200	0.300	0.400	0.500	0.700	1.000	1.500	2.000	3.000
ADRENALS	0.2811E-05	0.2663E-05	0.2813E-05	0.2622E-05	0.2671E-05	0.2930E-05	0.2525E-05	0.2587E-05	0.2377E-05
BLADDER WALL	0.1178E-06	0.1374E-06	0.1646E-06	0.1936E-06	0.2078E-06	0.2488E-06	0.3060E-06	0.3181E-06	0.3540E-06
BRAIN	0.4279E-05	0.4477E-05	0.4594E-05	0.4657E-05	0.4690E-05	0.4626E-05	0.4403E-05	0.4201E-05	0.3820E-05
EYES	0.5413E-05	0.5639E-05	0.5875E-05	0.5820E-05	0.5879E-05	0.5871E-05	0.5675E-05	0.5230E-05	0.4786E-05
HEART	0.1260E-04	0.1253E-04	0.1256E-04	0.1246E-04	0.1213E-04	0.1170E-04	0.1091E-04	0.1022E-04	0.9149E-05
LARGE INTESTINE WALL	0.7534E-06	0.7764E-06	0.8292E-06	0.8853E-06	0.9300E-06	0.9760E-06	0.9871E-06	0.9565E-06	0.9501E-06
SMALL INTESTINE WALL+C.	0.5107E-06	0.5609E-06	0.6086E-06	0.6475E-06	0.6927E-06	0.7438E-06	0.7798E-06	0.7820E-06	0.7671E-06
KIDNEYS	0.1158E-05	0.1221E-05	0.1270E-05	0.1303E-05	0.1358E-05	0.1378E-05	0.1374E-05	0.1322E-05	0.1258E-05
LIVER	0.3295E-05	0.3317E-05	0.3336E-05	0.3372E-05	0.3357E-05	0.3289E-05	0.3091E-05	0.2963E-05	0.2714E-05
LUNGS	0.1829E-04	0.1832E-04	0.1811E-04	0.1796E-04	0.1727E-04	0.1644E-04	0.1497E-04	0.1394E-04	0.1236E-04
OVARIES	0.3168E-06	0.3926E-06	0.4137E-06	0.3570E-06	0.4339E-06	0.5741E-06	0.7107E-06	0.4928E-06	0.5760E-06
PANCREAS	0.2024E-05	0.2083E-05	0.2072E-05	0.2084E-05	0.2120E-05	0.2140E-05	0.2055E-05	0.2030E-05	0.1894E-05
SKIN	0.3931E-05	0.4157E-05	0.4257E-05	0.4285E-05	0.4251E-05	0.4133E-05	0.3883E-05	0.3669E-05	0.3312E-05
SPINAL CORD	0.1437E-04	0.1444E-04	0.1434E-04	0.1416E-04	0.1382E-04	0.1336E-04	0.1221E-04	0.1165E-04	0.1023E-04
SPLEEN	0.2479E-05	0.2503E-05	0.2573E-05	0.2610E-05	0.2566E-05	0.2544E-05	0.2436E-05	0.2330E-05	0.2136E-05
STOMACH WALL	0.1854E-05	0.1959E-05	0.2071E-05	0.2081E-05	0.2107E-05	0.2139E-05	0.2062E-05	0.2043E-05	0.1939E-05
THYMUS	0.1225E-03	0.1252E-03	0.1262E-03	0.1257E-03	0.1213E-03	0.1161E-03	0.1060E-03	0.9891E-04	0.8702E-04
THYROID	0.2046E-02	0.2172E-02	0.2213E-02	0.2217E-02	0.2174E-02	0.2079E-02	0.1895E-02	0.1745E-02	0.1536E-02
TISSUE	0.6117E-05	0.6304E-05	0.6367E-05	0.6369E-05	0.6249E-05	0.6019E-05	0.5585E-05	0.5220E-05	0.4666E-05
UTERUS	0.2075E-06	0.2216E-06	0.2673E-06	0.2853E-06	0.3152E-06	0.3917E-06	0.4151E-06	0.4465E-06	0.3960E-06
SKELETON	0.8812E-05	0.7366E-05	0.6880E-05	0.6639E-05	0.6319E-05	0.5989E-05	0.5537E-05	0.5183E-05	0.4684E-05
RED BONE MARROW	0.6318E-05	0.6546E-05	0.6593E-05	0.6612E-05	0.6463E-05	0.6242E-05	0.5792E-05	0.5393E-05	0.4851E-05
TOTAL BODY	0.6669E-05	0.6723E-05	0.6745E-05	0.6731E-05	0.6591E-05	0.6349E-05	0.5891E-05	0.5512E-05	0.4936E-05
STOMACH CONTENTS	0.1869E-05	0.1966E-05	0.1993E-05	0.2052E-05	0.2085E-05	0.2123E-05	0.2103E-05	0.2043E-05	0.1923E-05
TESTES	0.3794E-07	0.5902E-07	0.4483E-07	0.7931E-07	0.8531E-07	0.7313E-07	0.1761E-06	0.1501E-06	0.1311E-06

Table 2: Dose equivalents per unit activity administered ($Sv.Bq^{-1}$) resulting from the incorporation of various radionuclides calculated with a voxel phantom and two mathematical MIRD phantoms

Nuclide	Tissue	5 yr old MIRD	7 yr old voxel	10 yr old MIRD
^{123}I thyroid uptake 15%	ovaries	3.8E-11	3.1E-11	2.5E-10
^{131}I thyroid uptake 25%	thyroid	1.9E-06	1.3E-06	8.4E-07
$^{99}Tc^{m}$ without blocking the thyroid	thyroid	1.2E-10	7.5E-11	5.6E-11

Table 3: Specific Absorbed Fractions (SAF) of photon energy in 1/kg, for a 5 year old child (19 kg)*, a 7 year old child (21.7 kg)** and a 10 year old child (32 kg)*

Source Organ : THYROID

Target Organ	Energy [MeV] 0.030			0.100			1		
	5 yr	7 yr	10 yr	5 yr	7 yr	10 yr	5 yr	7 yr	10 yr
ADRENALS	2.2E-04	4.1E-04	3.6E-05	3.0E-03	2.9E-03	1.2E-03	3.2E-03	2.9E-03	1.8E-03
BRAIN	2.9E-03	1.2E-03	1.9E-03	9.8E-03	4.0E-03	8.4E-03	9.9E-03	4.6E-03	8.9E-03
LIVER	6.7E-04	1.2E-03	1.9E-04	3.2E-03	3.5E-03	1.5E-03	3.1E-03	3.3E-03	1.7E-03
LUNGS	1.9E-02	3.8E-02	8.0E-03	1.7E-02	2.0E-02	1.1E-02	1.3E-02	1.6E-02	8.2E-03
OVARIES	1.3E-06	3.7E-06	6.5E-08	2.9E-04	3.1E-04	7.7E-05	7.2E-04	5.7E-04	3.3E-04
PANCREAS	2.0E-04	2.5E-04	2.8E-05	2.6E-03	2.1E-03	1.1E-03	3.0E-03	2.1E-03	1.7E-03
THYROID	2.4E+01	1.1E+01	1.4E+01	4.6E+00	1.9E+00	2.6E+00	5.4E+00	2.1E+00	3.0E+00
SKELETON	3.6E-02	4.4E-02	2.2E-02	2.6E-02	1.8E-02	1.7E-02	9.0E-03	6.2E-03	6.4E-03
RED BONE MARROW	5.4E-03	5.8E-03	3.3E-03	7.9E-03	5.8E-03	5.7E-03	6.6E-03	5.4E-03	5.0E-03

* Data of Cristy and Eckerman, ORNL, obtained using MIRD type mathematical phantoms (2).
** Data obtained from the present study, using a voxel phantom.

Head of project 4: Dr. Evans.

II. Objectives for the reporting period

The first objective for the reporting period January 1st 1993 to August 31st 1993 was to collect biokinetic data on 15 children after the administration of Tc-99m-DMSA, calculate the time integrated activity in the kidneys, liver, spleen, bladder and whole body and using these as source organs, estimate the equivalent radiation doses to 25 target organs, the Effective Doses (E) and the Effective Dose Equivalents (He) A further objective was to assess the diagnostic quality of imaging as a function of the amount of activity administered in children undergoing Tc-99m-MAG3 scans.

Due to the fact that the dosimetry study involves 8 visits for each child over a period of two days, it was difficult to get full cooperation from the children or the parents. Consequently our objective for the next reporting period is to do as many children as is possible irrespective of age. We hope to obtain radiation doses on a further 15 children and to show how these vary with age and kidney function.

A further objective in the next reporting period, is to set up criteria for the reproducibility of the clinical interpretation of Tc99m-DMSA scans and to construct a classification so that a clinical museum can be developed.

III. Progress achieved including publications

12 children between the ages of 3 weeks and 14 years have now been fully studied to obtain radiation dose estimates after the administration of Tc-99m-DMSA.

A brief description of the method is as follows:-
The dose injected was reduced according to patient weight based on an adult dose of 100MBq. It was then measured on the gamma camera and through the patient over the kidneys, bladder and knees. After the injection, the residue was measured and the anterior and posterior views of the patient were taken at intervals up to 30 hours. Also measurements were taken of the activity in the whole body both directly, using the gamma camera and indirectly by measurement of the urine excreted. Special software has been written to acquire and to process this data so obtaining the mid-plane activity of the injected dose and absolute uptake into the kidneys, liver, spleen, bladder and knees.

Dedicated software has been written to obtain time/activity plots and residence times in the source organs. The MIRDOSE 2

programme was then used to obtain dose estimates in 25 target organs from the 5 source organs. A dynamic bladder model was used assuming a 2 hour voiding period and a single clearance through the kidneys, to obtain the bladder residence time. The Effective Dose (E) and the Effective Dose Equivalent (He), were calculated.

The results of the first 12 patients are shown in table 1. The residence times do not change significantly with weight in patients with normal uptake of Tc-99m-DMSA by the kidney. The equivalent kidney dose, Effective Dose and Effective Dose Equivalent, in mSv/MBq are inversely related to the weight of the child. In the three children under 6 months, the Effective Dose was approximately 8E-2mSv/MBq, whereas in children over 5 years of age this dose reduced to approximately 1.5E-2mSv/MBq. In the child with very poor renal function, the Effective Dose was lower than expected for that age group and was due to the low uptake into the kidney and rapid clearance from the body as seen in the high output of Tc-99m-DMSA in the urine.

The activity remaining in the body was also calculated by measuring the activity excreted in the urine. The results from the urine analysis were very similar to those from direct measurement of the activity in the whole body.

The individual Effective Doses and equivalent kidney doses were also calculated. These are the doses received by the individual patients and are expressed in mSv. These doses are dependent on the activity injected which is reduced according to the weight of the patient. If this weighting factor is correct, then the individual Effective Doses should be similar for all the children irrespective of weight. The table shows that these Effective Doses and kidney doses vary from 0.74mSv to 1.52mSv and 12.6mSv to 30.4mSv. respectively, with no general trend. This could be due to there being only five models available for the MIRDOSE 2 calculations, i.e. neonate, 1 year old, 5 year old, 10 year old and 15 year old. A neonate model gives estimated doses a factor of 2.12 times higher than if a 1 year old model is used. Similarly, the factor for a 1 to 5 year old is 1.72, for a 5 to 10 year old is 1.47 and for a 10 to 15 year old is 1.52. Work is required to be done to obtain some form of linear interpolation between these models.

The radiation dosimetry study has been submitted as work in progress, for oral presentation at the European Association of Nuclear Medicine Congress 1993 in Lausanne.

The assessment of the diagnostic quality of imaging as a function of the amount of activity administered to children undergoing Tc-99m-MAG3 scans, has been completed and the results published in the Journal of Nuclear Medicine, 1992;33,2090-2093. When the sensitivity and specificity of the clearance image of MAG3 was compared with Tc-99m-DMSA scans in the detection of renal scars, there was no difference between those children who received a dose based on an adult schedule of 80 MBq from those receiving doses based on an adult schedule of 112MBq. scaled on a body surface area.

Publications

Evans, K. et al. Radiation Dosimetry of Tc-99m DMSA in Children.
European Association of Nuclear Medicine Congress 1993. Submitted for oral presentation.

Gordon, I. et al. Can Tc-99m-MAG3 replace Tc-99m-DMSA in the Exclusion of a Focal Renal Defect?
J. Nucl. Med., 1992;33,2090-2093.

Patient	E.S.	G.H.	H.R.	W.B.	D.L.	S.W.	S.L.	F.W.	T.C.	D.H.	V.S.	J.E.
Age (Yrs)	6.1	12.8	3 weeks	0.4	0.4	2.7	5.1	7	1	11	14.9	1 3
Weight (Kg)	16.5	41	4.2	4	6 6	16.2	21.4	32	7.6	65	80	8.02
Max. % kidney uptake (L/R)	0/44	24.1/22.4	18.1/18.3	19.4/19.6	23 0/19.1	0/5.1	18.1/12.1	1.3/17.8	23.5/25 9	22 9/24.2	24.2/25.2	19 5/18.3
% Global Uptake	44	46.5	36.4	39	42.1	5.1	26.2	19.1	49.4	47.1	49.4	37 8
Dose (MBq)	42	77	14	14	20	41	49	65	22	98	100	23
Source organ residence times (Hours)												
Kidneys	3 1	3.58	2.9	2.79	2 91	0.183	1.8	1.55	3.56	3.26	3.64	2.64
Liver	0.31	0.13	0.1	0.57	0.53	0.291	0.171	0.229	0.4	0.44	0.38	0.23
Spleen	0.03	0.05	0.06	0.11	0.09	0.131	0.034	0.168	0.2	0.27	0 21	0.05
Bladder	0.073	0.026	0 048	0.054	0.045	0.115	0.042	0.073	0.01	0 071	0.056	0.102
Rem. Body	4.14	4.32	4.84	4 35	4 61	5.19	4.96	5.76	4.1	3 47	3 52	4.34
Equivalent kidney dose (mSv/MBq)	3 67E-01	2.13E-01	1 51	1.46	1.52	2.76E-02	2.15E-01	1.88E-01	7.36E-01	2.70E-01	2.16E-01	5.47E-01
Effective Dose (mSv/MBq)	2.94E-02	9 94E-03	7.73E-02	8.13E-02	7.44E-02	1.03E-02	1.51E-02	1.69E-02	3 87E-02	1.55E-02	1 22E-02	3.79E-02
Effective Dose Equivalent (mSv/MBq)	5.72E-02	1.85E-02	0.138	1 44E-01	1.43E-01	1.26E-02	2.37E-02	2.64E-02	6.75E-02	2.70E-02	1.98E-02	5 32E-02
Effective Dose (mSv)	1 23E+00	7.60E-01	1.08E+00	1.14E+00	1.49E+00	4.40E-01	7.40E-01	1.10E+00	8.50E-01	1 52E+00	1 22E+00	8 70E-01
Equivalent kidney dose (mSv)	1.54E+01	1.64E+01	2.11E+01	2.04E+01	3 04E+01	1 00E-01	1.05E+01	1.22E+01	1 62E+01	2 65E+01	2 16E+01	1.26E+01

Table 1

patient	E.S.	G.H.	H.B.	W.B.	B.L.	S.W.	S.L.	E.W.	T.C.	D.H.	V.S.	J.E.
Date Study	07/09/92	14/09/92	26/01/93	22/03/93	30/03/93	18/05/93	18/05/93	25/05/93	01/06/93	15/06/93	29/06/93	06/07/93
Date Birth	14/08/86	20/11/79	04/01/93	19/10/92	11/11/92	30/08/90	26/03/88	20/05/86	17/06/92	28/06/82	19/09/78	28/03/92
Age (Yrs)	6.067077	12.8274	3 weeks	0.421918	0.380822	2.717808	5.147945	7.0191781	0.956164	10.9726	14.7863	1.2739726
Height (cm)	111	168	57	57	61	100	115	122	64	155	179	76
Weight (Kg)	16.5	41	4.2	4	6.6	16.2	21.4	32	7.6	65	80	8.02
Sex	F	M	M	F	M	M	M	F	F	M	F	M
Organ Doses												
(mGy/MBq)												
Adrenals	3.20E-02	1.58E-02	1.04E-01	1.04E-01	1.08E-01	1.14E-02	2.28E-02	2.29E-02	6.33E-02	2.31E-02	1.63E-02	4.97E-02
Brain	6.25E-03	2.48E-03	2.96E-02	2.67E-02	2.82E-02	7.80E-03	7.47E-03	8.67E-03	1.10E-02	3.26E-03	2.02E-03	1.16E-02
Breasts	5.76E-03	2.45E-03	2.78E-02	2.69E-02	2.81E-02	6.00E-03	6.10E-03	7.03E-03	1.14E-02	3.38E-03	2.18E-03	1.09E-02
GB Wall	2.14E-02	9.97E-03	5.88E-02	6.53E-02	6.69E-02	1.30E-02	1.72E-02	1.86E-02	3.35E-02	1.44E-02	1.05E-02	2.66E-02
LLI	1.09E-02	4.57E-03	4.22E-02	3.97E-02	4.15E-02	1.06E-02	1.11E-02	1.26E-02	1.93E-02	6.41E-03	4.08E-03	1.93E-02
Small Int	1.53E-02	7.12E-03	5.55E-02	5.44E-02	5.66E-02	1.09E-02	1.36E-02	1.49E-02	2.77E-02	1.01E-02	6.78E-03	2.47E-02
Stomach	1.44E-02	7.20E-03	4.86E-02	4.96E-02	5.13E-02	1.05E-02	1.27E-02	1.44E-02	2.51E-02	1.12E-02	7.33E-03	2.15E-02
ULI	1.50E-02	7.08E-03	5.02E-02	5.07E-02	5.26E-02	1.12E-02	1.35E-02	1.49E-02	2.60E-02	9.72E-03	6.79E-03	2.31E-02
Heart Wall	1.02E-02	4.60E-03	4.00E-02	4.01E-02	4.18E-02	9.43E-03	1.00E-02	1.15E-02	1.86E-02	6.70E-03	4.43E-03	1.70E-02
Kidneys	3.67E-01	2.13E-01	1.51	1.46	1.52	2.76E-02	2.15E-01	1.88E-01	7.36E-01	2.70E-01	2.16E-01	5.47E-01
Liver	2.07E-02	7.99E-03	4.54E-02	9.92E-02	9.59E-02	1.34E-02	1.37E-02	1.55E-02	4.11E-02	1.78E-02	1.15E-02	2.83E-02
Lungs	9.46E-03	4.24E-03	3.99E-02	3.96E-02	4.12E-02	8.64E-03	9.30E-03	1.06E-02	1.86E-02	6.06E-03	3.98E-03	1.68E-02
Muscle	8.95E-03	4.21E-03	3.93E-02	3.75E-02	3.93E-02	7.85E-03	8.75E-03	9.84E-03	1.73E-02	5.62E-03	3.87E-03	1.62E-02
Ovaries	1.19E-02	4.97E-03	4.81E-02	4.58E-02	4.78E-02	1.08E-02	1.17E-02	1.32E-02	2.15E-02	6.80E-03	4.45E-03	2.08E-02
Pancreas	2.24E-02	1.14E-02	7.03E-02	7.28E-02	7.49E-02	1.25E-02	1.76E-02	1.96E-02	4.25E-02	1.77E-02	1.13E-02	3.35E-02
Red Marrow	1.08E-02	5.57E-03	3.99E-02	3.74E-02	3.93E-02	8.85E-03	1.03E-02	1.15E-02	1.86E-02	7.36E-03	5.16E-03	1.74E-02
Bone surface	1.56E-02	7.56E-03	6.43E-02	6.17E-02	6.45E-02	1.32E-02	1.51E-02	1.68E-02	3.12E-02	1.00E-02	6.89E-03	2.84E-02
Skin	5.73E-03	2.40E-03	2.84E-02	2.68E-02	2.81E-02	5.76E-03	6.03E-03	6.86E-03	1.12E-02	3.31E-03	2.12E-03	1.09E-02
Spleen	3.18E-02	1.67E-02	1.43E-01	2.05E-01	1.82E-01	4.23E-02	2.54E-02	6.14E-02	1.39E-01	6.52E-02	2.86E-02	5.73E-02
Testes	6.52E-03	2.68E-03	3.10E-02	2.86E-02	3.00E-02	7.92E-03	7.36E-03	8.63E-03	1.18E-02	3.49E-03	2.26E-03	1.29E-02
Thymus	7.49E-03	3.31E-03	3.39E-02	3.17E-02	3.34E-02	8.35E-03	8.33E-03	9.59E-03	1.35E-02	4.21E-03	2.84E-03	1.36E-02
Thyroid	7.77E-03	3.07E-03	3.54E-02	3.24E-02	3.42E-02	9.27E-03	9.02E-03	1.04E-02	1.40E-02	4.05E-03	2.53E-03	1.44E-02
UB wall	1.52E-02	4.97E-03	5.25E-02	5.40E-02	5.09E-02	2.03E-02	1.23E-02	1.68E-02	1.61E-02	9.45E-03	5.96E-03	3.44E-02
Uterus	1.16E-02	4.80E-03	4.68E-02	4.46E-02	4.64E-02	1.15E-02	1.15E-02	1.33E-02	1.98E-02	6.92E-03	4.36E-03	2.06E-02
Total body	1.19E-02	5.35E-03	4.92E-02	4.91E-02	5.10E-02	8.79E-03	1.07E-02	1.18E-02	2.32E-02	7.58E-03	5.12E-03	2.03E-02

Progress Report

Contract: **F13P-CT920064g** **Sector: C22**

Title: Medical dose assessment and evaluation of risk.

1)	Wall	NRPB
2)	Drexler	GSF
3)	Fitzgerald	Hosp. St. Georges
4)	Zoetelief	TNO

I. Summary of Project Global Objectives and Achievements

Objectives

The contract combines a number of projects concerned with radiation dosimetry and quality control in diagnostic radiology aimed at assessing radiation doses and risks to patients and reducing them without compromising the diagnostic value of the examinations. The objectives are to use Monte Carlo organ dose calculations to improve the ease and accuracy with which doses to patients from x-ray examinations can be estimated and to develop a common basis for patient dosimetry and risk evaluation within the European Community. Improved quality control of radiological procedures is being encouraged by the implementation of an EN 29000 (BS 5750) Quality Management System in a radiology department and by providing dosimetric and technical advisory support to the development and testing of CEC Working Documents on Quality Criteria for Diagnostic Radiographic Images. Particular attention is paid to meeting these objectives in the fields of paediatric radiology and mammography.

Achievements

Considerable progress has been made in developing and extending Monte Carlo organ dose calculations at NRPB, GSF and TNO. Organ doses and effective dose normalised to both entrance surface dose and dose-area product have been calculated at NRPB using an improved mathematical phantom representing an adult patient and now cover 68 radiographic projections each with a choice of 40 x-ray spectra. Construction of a more realistic voxel adult phantom continues at GSF and organ dose calculations for paediatric CT examinations have been made using their existing BABY and CHILD voxel phantoms. These have been combined with field studies in German Childrens' Hospitals of the free-in-air axial doses under typical clinical CT conditions. St George's Hospital is collecting information on the radiographic procedures adopted during all common types of paediatric x-ray examination in the UK which will be used by NRPB to calculate organ doses using Monte Carlo procedures with a series of paediatric mathematical phantoms. These results will eventually be compared with those of similar calculations by GSF using their voxel phantoms and simulating radiographic conditions prevalent in Germany.TNO has used Monte Carlo calculations to estimate coefficients for converting air kerma free-in-air to average glandular tissue dose in mammography. The studies have concentrated on assessing the influence of breast composition and the x-ray spectrum on the conversion coefficients.

The relationship between radiation dose and health detriment for medical exposures has been studied by NRPB particularly with respect to the influence of the age of the patient at exposure. A twofold increase in risk for children compared to the average for the whole population, and a greater than fivefold reduction for the elderly, has been demonstrated.

Good progress has been made in the implementation of a BS 5750 Quality Management System in the x-ray department of a children's hospital by the St George's team. A Steering Committee has been set up, policy and procedural aspects of the quality manual are nearly complete and work instructions are being drafted. Detailed studies of paediatric radiology practice and measurements of image quality and patient dose are also underway at five hospitals. Further work on the optimisation of paediatric radiology includes the supply and readout of dosemeters for a European trial of the CEC paediatric quality criteria document by GSF and NRPB. Both these groups have provided technical advice on a report of the 1991 trial of the CEC adult quality criteria document.

Head of project 1: Mr Wall

II. Objectives for the reporting period

1. Extension of Monte Carlo calculations of organ doses for x-ray examinations on adult patients to cover more projections, more organs, more dose quantities and commence similar calculations for paediatric patients.

2. Provide dosimetry and technical advisory support for European trials of CEC Quality Criteria for Diagnostic Radiographic Images.

3. Assess radiation risks from medical x-rays.

Objectives for the next reporting period

1. Monte Carlo calculations of organ doses from common x-ray examinations on paediatric patients will be continued and results compared with those of GSF where appropriate.

2. Support to the revision of the CEC Working Document on Quality Criteria for Diagnostic Radiographic Images as a consequence of the results of the 1991 CEC trial.

3. As a further development of age specific risks for medical exposures, a link with other measures of health detriment (or benefit) such as Years of Life Lost (YOLLs) or Quality Adjusted Life Years (QALYs) will be sought.

III. Progress achieved including publications

1. An oesophagus has been modelled in the mathematical phantom representing an adult patient and our Monte Carlo organ dose calculations have been extended to include all the organs required to calculate the newly defined quantity 'effective dose'. The calculations now include 68 radiographic projections, each with 40 x-ray spectra allowing a wider range of examinations to be simulated. These projections include those used during complex examinations involving fluoroscopy, such as barium meals, barium enemas and cardiac angiography. Organ doses have been normalised to both entrance surface dose and dose-area product. Effective doses normalised to these two easily measurable dose quantities have also been calculated for the 68 radiographic projections and to dose-area product alone for 9 types of complete x-ray examination, thus providing a quick and reliable method for converting measurable patient doses into effective dose. A detailed study of barium meal and barium enema examinations at local hospitals was necessary to determine the influence of the wide variations in examination technique adopted by different radiologists.

   In collaboration with St George's Hospital, x-ray fields and exposure conditions representative of routine paediatric radiography have been defined and will be used in the Monte Carlo program to simulate x-ray examinations on a range of geometric phantoms of different sizes representing patients from newborn to 15 years old. Diagrams of these paediatric phantoms have been prepared to aid in the selection of appropriate irradiation geometries. The corresponding normalised organ doses and effective doses will be calculated to provide improved data for estimating radiation risks to children.

2. Advice and support have been provided on the dosimetric aspects of two initiatives that are being developed by Study Groups set up from within the group of contractors working on the optimisation of medical exposures in the CEC Radiation Protection Research Programme. These two initiatives involve the performance and evaluation of European-wide trials of quality criteria for diagnostic radiographic images in paediatric and adult radiology respectively. Together with the Children's Hospital

of the University of Munich, GSF and USL Udine a second trial of the CEC paediatric quality criteria document has been started with NRPB providing TL dosemeters to 25 hospitals in the UK, Ireland and Scandinavia.

Detailed comments have been made on a report from CAATS, Paris, on the results of the 1991 trial of the CEC adult quality criteria document. NRPB and CAATS have prepared a Refresher Course on the subject of this CEC document which will take place at the European Congress of Radiology in Vienna on 15 September 1993.

3. The 1990 recommendations of the ICRP, together with the latest epidemiological data and radiation risk models, have been studied to evaluate the risks to patients from x-ray examinations. The new ICRP concept of aggregated detriment and the corresponding definition of effective dose has been considered, with regard to their possible application to medical exposures. In particular the influence of age at exposure on risk coefficients and on the definition of effective dose has been assessed, to devise a simple system to account for these factors when estimating radiation detriment to patients and particularly to children. As a first step it has been decided that effective dose can, in most cases in which several organs are exposed, be used to assess the detriment from medical exposures, but it should be recognised that it will underestimate the detriment from paediatric exposure by about a factor of two and overestimate that from geriatric exposure by at least a factor of five. These ideas are expressed in the first three publications listed below.

Publications

Wall, B F, 1992. Risk assessment for the medical use of ionising radiation. Annales de l'Association Belge de Radioprotection, Vol 17, no. 2-3. p. 89.

Wall, B F, 1993. Risks of Imaging. Proc. R. Coll. Physicians Edinb. vol. 23, 164-169.

Shrimpton, P C, Wall, B F, Croft, J R and Webb, G A M, 1993. Medical Exposure. Guidance on the 1990 Recommendations of the ICRP. Docs. of the NRPB, Vol. 4, No. 2 (London, HMSO).

P C Shrimpton, 1992. Protection of the Patient in X-ray Computed Tomography. Doc. NRPB, Vol 3, No. 4, (London, HMSO).

IPSM/NRPB/CoR (Wall), 1992. National Protocol for Patient Dose Measurements in Diagnostic Radiology. (Chilton, NRPB).

Wall, B F and Hart, D, 1992. The potential for dose reduction in diagnostic radiology. Radiation Protection Dosimetry, Vol. 43. No. 1/4, pp 265- 268.

Shrimpton, P C and Wall, B F, 1992. Assessment of patient dose from computed tomography. Radiation Protection Dosimetry, Vol. 43. No. 1/4, pp 205- 208.

In press

Hart, D, Jones, D G and Wall, B F, 1993. Estimation of effective dose in diagnostic radiology from entrance surface dose and dose-area product measurements. NRPB-R262 (HMSO, London)

Head of project 2: **Dr Drexler**

II. Objectives for the reporting period

1. Construction of a voxel phantom on the basis of CT-data of a real adult patient. This work aims also to develop an automated segmentation technique, which up to now does not exist for low contrast contours and extended body regions.

2. Calculation and compilation of organ doses from paediatric CT-examinations using existing BABY and CHILD phantoms.

3. Field study to evaluate doses from paediatric CT-examinations of skull, thorax, abdomen and spine (together with the Childrens Hospital of the University of Munich).

4. Field study to evaluate doses from eight paediatric examinations of five year old children at 150 European hospitals (together with the Childrens Hospital of the University of Munich, NRPB and USL Udine).

Objectives for the next reporting period

1. The construction of voxel phantoms will be continued.

2. The calculation of organ doses from medical examinations will be continued.
   - Organ doses from examinations performed with radiographic techniques recommended in the CEC Working Document on Quality Criteria for Diagnostic Images in Paediatrics.
   - Relation between dose-area-product and organ doses for common radiographic examinations.

3. Dose measurements and field studies in routine X-ray examinations to support coordinated CEC-projects and initiatives.

4. Support to the revision of the CEC Working Document on Quality Criteria for Diagnostic Radiographic Images as a consequence of the results of the 1991 CEC trial.

III. Progress achieved including publications

1. Construction of Phantoms

The construction of a realistic phantom on the basis of whole body CT-data from an adult male patient was continued. Such phantoms offering an improved anatomical description of a patient (bone marrow, head) are necessary to verify the numerous results which were achieved up to now by means of mathematical MIRD type phantoms and to study the impact of phantom size and phantom anatomy on the determination of dose to patients.

So far the patient couch, the outer contours of the patient, the adipose tissue, the muscles and the skeleton were segmented. Separation of adipose and muscle tissue brings a new addition to phantom characteristics. The segmentation was performed by newly developed algorithms for the various organs and tissues avoiding manual interference. This research work is necessary because complete segmentation programs for routine use exist up to now only for restricted body regions containing high contrast objects (hip joints, contrast media filled vessels etc).

2. Calculation of organ dose

For the voxel phantoms BABY and CHILD organ doses were calculated resulting from any 1 cm CT-slice across the whole body. This was done for two radiation qualities (80 kV, 2.2 mm Al + 0.2 mm Cu and 125 kV, 2.2 mm Al + 0.2 mm Cu) and scanning angles of 360° and 180°. For CHILD the consequences of eccentric (6 cm) positioning of the patient were examined. Differences from the situation when the phantom was centrally positioned. were detectable but small compared with those resulting from other discrepancies between a real and the simulated situation. Furthermore, for CHILD the influence of asymmetric fan beams was calculated (25 cm + 10 cm instead of 25 cm + 25 cm in focus to axis distance). Such beams lead to markedly lower doses in the parts of the phantom outside the 10 cm field. For red bone marrow in the clavicles and the scapulae the doses are reduced by 9% - 10%, for the red marrow in the arm bones the reduction amounts to 24%.

All data were compiled in a catalogue of organ doses.

One weakness of the calculation of absolute organ doses, using the adult MIRD phantoms ADAM and EVA was corrected. In former calculations the entrance and exit dose was determined as the average dose across an area of the surface of the phantom which is projected to an area of 10 cm * 10 cm perpendicular to the axis of the central beam. In the case of the lateral examinations of lumbar and thoracic spine with the central axis of the beam outside the axis of the phantom, this caused a severe overestimation of exit dose and consequently a severe underestimation of organ entrance doses since the ratio of exit dose and entrance dose is used in the calculation. Now the width of this area is reduced to 4 cm, thus resulting in realistic exit surface and organ doses.

3. Dosimetric supply to CEC initiatives

In co-operation with the Childrens Hospitals of the Universities of Munich and Würzburg a field study was performed to evaluate doses free in air on the axis of rotation for typical paediatric CT-examinations of skull, thorax, abdomen and spine at 50 facilities. Results (in mGy) from 20 facilities:

	Median	1st Quartile	3rd Quartile	Range
Skull	39	24	44.7	13.9 - 74.2
Thorax	33.1	29.4	59.9	13.0 - 95.5
Abdomen	31.7	20.3	52	15.2 - 94.7
Spine	53.8	37.9	73.4	11.5 - 13.2

Beside the wide spread of dose values and an often occurring discrepancy between nominal and real slice widths, in about 1/3 of the units a disproportionality between the mAs-value and doses (for the same kV setting, focus distance and filtration) was to be observed.

Together with the Childrens Hospital of the University of Munich, NRPB and USL Udine a second CEC trial was prepared and started to measure patient entrance doses for eight paediatric radiographic examinations at 150 European hospitals. 35 hospitals (from 70 to be evaluated by GSF) were up to now provided with TL-dosemeters and 15 returned them already.

Publications issued during the reporting period

Drexler, G. Das Konzept der Effektiven Dosis in der Röntgendiagnostik. In: Strahlenschutz in Forschung und Praxis, Band 34: Strahlenexposition bei neuen diagnostischen Verfahren (Hrsg: F. Holeczke et al) Stuttgart: Gustav Fischer Verlag, 1-14 (1993).

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Head of project 3: Mr Fitzgerald

II. Objectives for the reporting period

The project commenced in November 1992 and the objectives were:

1. Implementation of a BS 5750 Quality Management System in the X-ray Department at Queen Mary's Children's Hospital;

2. The study of paediatric radiology practice, patient doses and image quality in general in paediatric hospitals.

Objectives for the next reporting period

1. Analysis of data already obtained together with a continuation of the studies listed above;

2. A visit by clinical staff of Queen Mary's Hospital, Carshalton to the Children's Hospital of the University of Munich;

3. Extension of the project to include CT and Neo-natal radiology;

4. Completion of a Quality Manual.

III. Progress achieved including publications

Staff

A Senior Radiographer was appointed at the end of the October 1992 (0.4 WTE) and an Associate Physicist on 25 January 1993 (full time). Secretarial assistance is provided by an Agency when necessary. The Deputy Superintendent Radiographer at Queen Mary's Hospital for Children contributes 0.2 WTE in acting as the Quality Manager.

Hospitals included in the project

Queen Mary's Hospital for Children, Surrey (QMC)
Queen Elizabeth Hospital for Children, London
Queen Mary's University Hospital, Roehampton (General Hospital)
Mayday University Hospital, Croydon, (General Hospital)
Guy's Hospital (to be confirmed) (Teaching Hospital)

Organisation

A steering Committee meets monthly and the meetings are minuted. Membership is:

Mr M Fitzgerald, Consultant Physicist (Chairman)
Dr V Cooke, Consultant Radiologist (Secretary)
Dr S Pablot, Consultant Radiologist
Mrs K Shah, Superintendent Radiographer
Miss D Garner, Deputy Superintendent Radiographer and Quality Manager
Mrs A Pettett, Senior Radiographer
Mr J Kyriou, Associate Physicist

Frequency, Radiological Referral Criteria and
Radiological and Radiographic Practice and Technique

Fifteen of the commonest types of paediatric X-ray examinations had been selected, sub-divided into five age groups. For each of these frequency data has been collected at four of the above hospitals for two specific months and information on radiological referral criteria for undertaking examinations and radiological and radiographic practice and technique has been collected at three of the above hospitals. For QMC these have been written-up as part of the Quality Manual and a comparison with the information from other hospitals is to be made. The information has been collected by interviewing at least two radiographers at each centre and by discussion with the radiologists who undertake the bulk of the paediatric work.

Dose Measurements using TLDs and Dose-Area Product Meters

The dosimetry of over 100 patients, covering a number of routine examinations at two of the designated hospitals has already taken place using TLDs. This work is continuing and will incorporate the other three hospitals. (ESDs have been produced and compared to other sources).

Dose-area product results have been collected for some 250 fluoroscopic examinations at one of the children's hospitals. This work is soon to be extended to incorporate the other designated hospitals.

ESDs have been calculated for a total of some 350 radiographic examinations using theoretical methods of calculation at one children's hospital. This work is soon to be extended to incorporate the other designated hospitals.

The Influence of Radiographic Technique on Patient Dose and Image Quality

Phantom studies have been undertaken to study the relationship between patient dose and image quality for the case of 1 year old chest imaging. The effects of varying tube potential, tube filtration and intensifying screen has been assessed. This work is continuing and will be expanded to incorporate other age groups and possibly other examinations.

Quality System

A quality manual is being written in four levels (a) Policy, (b) Procedures, (c) Work Instructions, (d) Records. Level 1 is complete and level 2 is well advanced, particularly on those aspects that are directly concerned with radiographic practice. Level 3 is drafted for fifteen types of examinations, including referral criteria and radiographic technique.

As part of the introduction of the quality system at QMC several studies are being undertaken in parallel. These include the effect on radiological referrals of the introduction of 'Guidance on Use of the X-ray Department' which has been written for use by referring clinicians. It is based on CEC criteria and the UK Royal College of radiologists Guidelines. It has been introduced to GP's and to junior medical staff at QMC through a number of seminars. In another study, the two radiologists at QMC are vetting all films and scoring them against a number of criteria based on CEC image quality criteria and also local criteria. The influence of the introduction of quality methods into the X-ray Department is being assessed at this stage.

Publications

Booklet - "Guidance on the Use of the X-ray Department" - for local use by referring clinicians, based on CEC criteria and UK RCR Guidelines.

Head of project 4: Dr Zoetelief

II. Objectives for the reporting period

Average absorbed dose in glandular tissue is the most appropriate quantity for risk assessment in mammography. Calculations will be made of air kerma free-in-air to average glandular tissue dose conversion factors, g, for simple mathematical breast models using the radiation transport code MCNP. For the calculations, spectra published by Birch et al (1979) and Panzer et al (1978) will be used. The influence of differences in elemental composition of breast composing tissues (Hammerstein et al, 1979 versus ICRU-44, 1989) and of differing superficial layers used to represent skin and underlaying adipose tissue will be studied.

Objectives for the next reporting period

National standards laboratories do not provide calibrations at real mammography qualities, although efforts have been made to provide calibration facilities at near mammography qualities. At St George's Hospital a calibration facility for mammography has been established which employs real mammography units and is therefore unique. A dosimetry intercomparison between TNO and St George's Hospital will be valuable to improve absolute dosimetry. The calculation of conversion factors, g, will be extended to study the influence of the presence of a compression plate, variations in breast thickness and differences in spectra with the aim of explaining differences in results obtained by different investigators.

III. Progress achieved including publications

For the calculation of air kerma free-in-air to average glandular tissue dose conversion factors, g, the Monte Carlo Neutron and Photon transport code system (MCNP) was used in which the photon cross section data of Berger and Hubbell (1987) were introduced. Photon spectra published by Birch et al (1979) and Panzer et al (1978) were employed. Half value layers (HVL) were calculated for these spectra behind a compression plate of 3 mm thickness made of polymethylmethacrylate (PMMA).

Mathematical standard breast models were employed consisting of a central region with a mixture of 50 per cent glandular tissue and 50 per cent adipose tissue of varying thickness surrounded by a superficial layer of adipose tissue of 0.5 cm thickness. The total phantom thickness was varied from 5.0 to 6.0 cm, since this is approximately the range of average breast thickness. Calculations were made at a fixed focus-to-backscatter plate distance of 60.0 cm. The backscatter plate consisted of 2.0 cm of PMMA. For some calculations the superficial layer of 0.5 cm of adipose tissue was replaced by 0.4 cm of glandular tissue, since this is also used to represent skin and underlying adipose tissue.

The density and the elemental compositions of glandular tissue and adipose tissue were determined by Hammerstein et al (1979) for five samples of fresh mastectomy specimens. Data of Woodard and White (1986) refer to glands embedded in, but separated from a large mass of hormonally controlled adipose tissue and are the means of seven specimens from post-menopausal women. According to White (private communication, 1991) the two sources of data on glandular tissue composition are essentially the same. As the specimens considered contained both "normal and abnormal" tissue, Woodard and White attempted to select only "normal" tissue. Therefore, the ICRU (1989) recommended the average value of Woodard and White as reference composition for glandular tissue. It should be noted, however, that the composition of Hammerstein et al is usually employed for dosimetry.

The results of calculations for 5.0 and 6.0 cm thick "standard" breasts are presented in the Table for calculations with photon spectra of Birch et al.

For 6 cm thick breasts, g-values show maximum differences of 13 per cent at an HVL of 0.30 mm Al with those of other investigators ie Dance, 1990. The maximum difference is reduced to 8 per cent at an HVL of 0.40 mm Al and refers to values of Wu et al, 1991. These differences concern the situation where all investigators use elemental compositions of Hammerstein et al.

Table: Conversion factors, g, for 5 and 6 cm thick "standard" breasts with tissue compositions according to Hammerstein et al and ICRU-44

HVL (mm Al)	Elemental composition of Hammerstein et al		Elemental composition of ICRU-44	
	5 cm thickness	6 cm thickness	5 cm thickness	6 cm thickness
0.30	0.154	0.124	0.135	0.108
0.35	0.182	0.147	0.159	0.128
0.40	0.212	0.173	0.186	0.151

It is clear from the Table that the differences due to elemental compositions of Hammerstein et al compared to ICRU-44 are considerable i.e about 14 to 15 per cent.

At an HVL of 0.35 a g-value of 0.152 is obtained for a 6 cm thick breast employing spectral data of Panzer et al and tissue compositions of Hammerstein et al compared to the value of 0.147 given in the Table for spectra from Birch et al. This indicates that specification HVL alone is not sufficient for characterising the radiation quality. This observation is in agreement with the results of Wu et al, which are showing differences of about 7 to 8 per cent for the same HVL depending on tube voltage.

The influence of the composition and thickness of the superficial layer representing skin and underlaying adipose tissue is dependent on the source used for elemental composition of breast composing tissues. Preliminary results using data from Hammerstein et al result in a difference of 17 per cent between superficial layers of 0.4 cm glandular tissue and 0.5 cm adipose tissue. Using data from ICRU-44 this difference is reduced to about 7 per cent. This requires further investigation.

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Publications

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Progress Report

Contract: **FI3P-CT930070** **Sector: C22**

Title: Evaluation of dose and risk due to interventional radiology techniques.

1)	Schmidt	Klinikum Nürnberg
2)	Maccia	CAATS
3)	Padovani	Unitá Sanitaria Locale - Udine
5)	Neofotistou	Athens General Hospital*
6)	Vañó Carruana	Universidad Complutense de Madrid*

I. Summary of Project Global Objectives and Achievements

The aim of the research project is to determine the radiation exposure of patient and staff due to interventional radiology.
The evaluation of the dose aims to give information about the extent to which interventional treatment may cause deterministic detriments to patients. With regard to stochastic detriments, the contribution of interventions to the collective dose equivalent will be determined. Due to the anticipated high exposure of the patient and the complex examination techniques, the radiation exposure of the staff will be determined, too.
Because of the anticipated results, a programme for an optimum minimizing of exposure intensity will formulated for both patients and staff.
Due to the different circumstances and prerequisites in the 5 participating countries the main emphasis of the research project of the different participants may vary, either complementing or overlaping that of the others.
Extensive enquiries about interventional examinations in general, or some aspects of them are planned in each of the countries. For example enquiries will help to ascertain how many procedures are performed, by specifying type and location. The influence of age and sex regarding the kind of intervention will be considered, as well as the equipment used. Another aspect will be how many radiologist perform interventional examinations and how many catheters are used per intervention, etc..
The most representative and relevant dosimetric parameters, such as dose area product, duration of fluoroscopy, and the number of frames, will be determined. Patient and staff dosimetry will be done separately, by sample.

* The Greek and Spanish participants only started recently their investigations, focused on quality control aspects.

Head of project 1: Prof. Schmidt

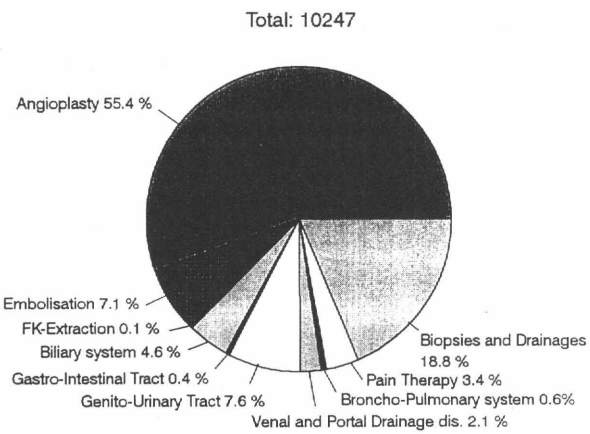
II. Objectives for the reporting period

The principal objectives for the reporting period were to gather information and statistics of interventional radiology practiced in Germany. Our intention is to initiate, organize and carry out a survey of interventional examinations. Additionally, an evaluation of data regarding the X-ray equipment, the number of patients and the staff involved was planned to be started in general hospitals, as well as the measurement of characteristic dose values of X-ray-equipment.

III. Progress achieved including publications

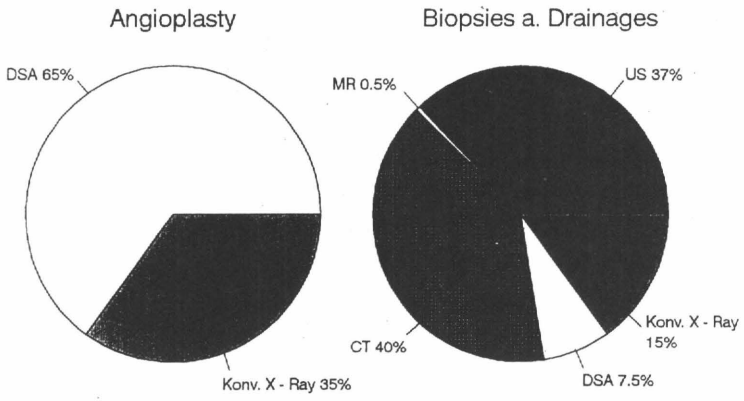
Owing to the different organizational structures in Germany, cardiological interventions have to be investigated separately. First inquiries in cardiology lead to an estimate of about 35,000 PTCAs per year in Germany, which corresponds to about 500 PTCAs per 1 million inhabitants. The frequency of PTCA can be calculated on the basis of the consumption of PTCA-catheters. In comparison to this, in the U.S. about 1,000 PTCAs were performed per year per

Fig.1: **Interventional Radiology 1992**



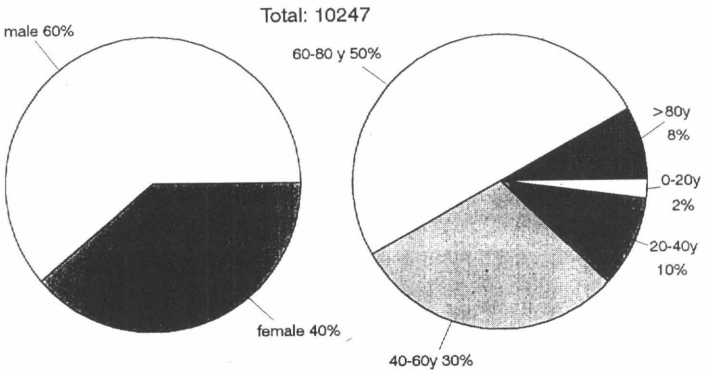
1 million inhabitants. Comments in the literature indicate that a great number of these PTCAs were not justified (commercial interests). Recent data from Finland report that about 200 PTCAs/1 million inhabitants were performed.
The first estimate of the total number of radiological interventions is, of course, a rough one. The anticipated frequency is about 1.000 to 3.000 interventions per 1 million inhabitants in Germany.
According to the data of more than 10,000 patients collected for this project, more than 50% of all radiological interventions in 1992 were angioplasties (see fig. 1). For this reason special attention has to be paid to PTA for further investigations. For different types of interventional examinations the portion of imaging using ionizing radiation is quite different. For example fig. 2 shows the different distribution of imaging techniques of the two most common types of intervention.

Fig.2: **Imaging**



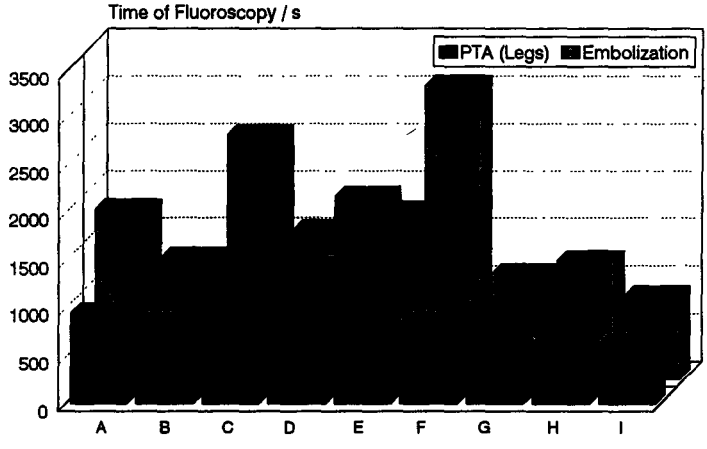
In addition to these data, patient-related data are essential to assess the stochastic detriment. Fig. 3 shows the sex and age distribution of patients, averaged over all types of interventions. It is surprising, that nearly 2/3 of all interventions are performed in males. Far more than 50% of all patients are older than 60 years. On the basis of these data, which have to be differentiated according to the type of intervention, and using risk values according to publication

Fig.3: **Sex- and Age-Distribution**



ICRP (60) and NRPB (Vol.4,No.2, 1993), there is a possibility for detailed calculation of the effective dose to the patient.
For this purpose, and the evaluation of dose values of the different types of interventions, 14 general hospitals in Germany were visited until September 1993. On the one hand data of frequency were collected and types of intervention, as well as characteristic dosimetric parameters of the X-ray equipment, and on the other hand dose measurements were carried out on phantoms. Fig. 4 shows the mean time of fluoroscopy of 2 kinds of interventions in 9 general hospitals all over Germany. It is remarkable how much the dose-relevant parameters vary between these hospitals, and that the variance changes with the kind of intervention.

Fig. 4: **Variance of Average Time of Fluoroscopy 9 German General Hospitals**



In accordance with the objectives of the project to make a survey on both the stochastic and the deterministic detriments, it is essential to determine the mean values of relevant dosimetric parameters, as well as the variance and its distribution. On the basis of such distributions it is possible to estimate the frequency of deterministic detriments due to interventional radiology.

Further collection and analysis of data and dosimetric measurements are planned.

Head of project 2 : Dr. Maccia

II. Objectives for the reporting period

The principal objectives for the reporting period were (1) to gather the most relevant information concerning the structure and the institutional framework within which the Interventional Radiology (IR) is practiced in France and (2) to organise and conduct a survey on the number and frequency of Interventional Radiology examinations performed.

III. Progress report achieved including publications

Considering the strong interest shown by the French Radiologists for the Interventional Radiology on one hand, and the large diffusion of such imaging technique on the other, a first tentative was made to identify the categories of medical specialities which actually perform Interventional Radiology examinations.
In order to cover all possible cases and get involved as many radiologists as possible, four separate postal surveys were therefore designed.

More than 800 questionnaires were sent to the following practitioner categories:

- University Teaching Hospital Radiologists (167 questionnaires) ;
- members of the French College of IR (244 questionnaires) ;
- radiologists working in the vascular radiology departments (295 questionnaires) ;
- radiologists working in the main hospital of the Parisian region (100 questionnaires).

Each questionnaire asked for a detailed list of organisational and technical items whch decribe the "on-site" structure of the IR : type and category of medical and paramedical personnel involved (radiologists, anesthesists, vascular surgeon etc.), age of patient (adult and/or pediatric), number and type of IR examinations yearly performed in 1992, type of radiological equipement used (angiographs, CT scanner, ultrasound, fluoroscopy, NMR).
Because of the address files inaccuracy and the complexity of the structure of the IR practice (the same practitioner may be involved in both private and

public sector radiological activity and therefore doubly counted) a certain number of questionnaires were not reliable for the final data analysis.
The actual response rate of the survey was therefore 25,8% (160 out of 620 reliable questionnaires).
It is already anticipated that such a lower figure will lead to an underestimation of the actual IR practice in France.
The very preliminary results of the survey extrapolated to the whole country are the following :

a) The practitioners :

The Figure 1 shows the percentage of Interventional Radiology centres where the examinations are performed exclusively by the radiologists, brokendown by the number of the radiologists actually involved.

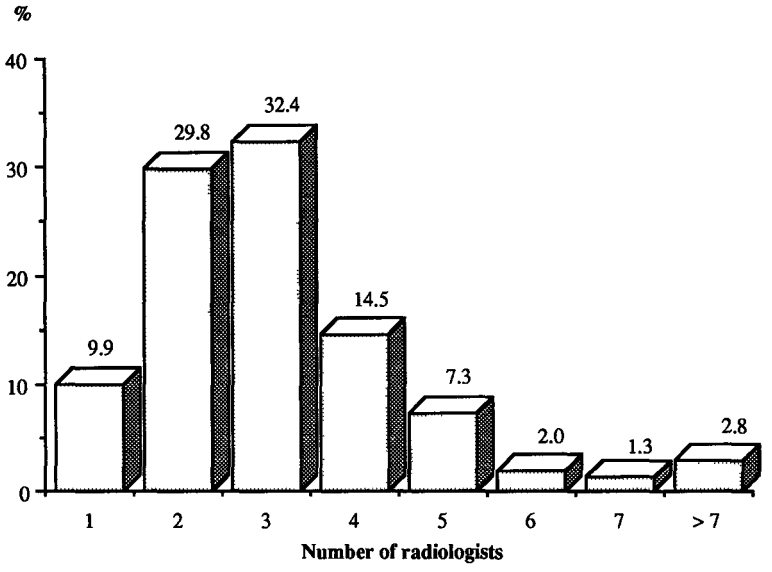


Figure 1 : Breakdown of Interventional Radiology examinations

One may observe that 3 radiologists per Interventional Radiology centre on average perform these examinations and that slightly more than 60% of IR centres have 2 or 3 radiologists working in this particular field.

b) The frequency of IR examinations :

I.R. DOMAINES	Number of examinations	%
VASCULAR Interventional Radiology		
Arterial and venous dilatation	10,693	18.8
Embolisation (not including neuroradiology)	2,220	3.9
Chemo-perfusion - chemo-embolisation	2,609	4.6
CARDIOLOGY Interventional R adiology	2,089	3.7
NEURORADIOLOGY Interventional R adiology		
Embolisation	1,718	3.0
Chemoperfusion	608	1.1
Revascularization head - neck	199	0.3
Stereotaxy	545	1.0
Pain treatment	395	0.7
Spine and vertebral canal	8,449	14.8
VISCERAL Interventional R adiology	18,680	32.8
OSTEO-ART. Interventional R adiology	8,737	15.3
TOTAL	**56,942**	**100**

Table 1 : Number and type of IR examination by medical specialisation category (France 1992).

Despite the general problem of terminology, five wide examination categories were defined in order to present the survey results.

As one may notice, approximately 57,000 IR examinations were carried out in France in 1992 among which the most frequent were the vascular examinations followed by the visceral examinations which include thoracic (percutaneous drainage, biopsy), digestive system (liver puncture, alcoholization of hepatic tumor) and uro-genital (urethroplasty, ureteral drainage) investigations.

As planned within the framework of the coordinated project, further analysis of technical collected data are under way in order to select the most relevant dosimetric parameters to be measured.

Supplementary effort will also be made to increase the number of participating practitioner categories involved in this study. This will permit to improve the overall statistical significance of data.

Head of project 3: Dr. Padovani

II. Objectives for the reporting period

Interventional Radiology (IR) represents one of the most recent application of modern imaging technology in radiology and in many cases it substitutes surgical intervention.
The project aims to evaluate type and frequency of neuroradiological interventional procedures performed in Italy.
In the second period of the study, patient and staff doses will be evaluated for each type of examination with dosimetric methods developed in collaboration with other European groups participating at the project.

III. Progress achieved including publications

For the evaluation of type and number of interventional procedure performed in neuroradiology in Italy, a questionnaire was developed in collaboration with Italian Association of Neuroradiology and in accordance with the other participants to the project.
The questionnaire was distributed to the Italian neuroradiological departments in June 1993 and collection of data will continue until December 1993.

The questionnaire is consists of two parts:
A) A part to be filled for each neuroradiological procedure with information on patient, patient pathology and radiological procedure performed (Angiography, Biopsy, Embolisation, Chemotherapy, Angioplasty, Nucleolysis and Mielography). For each procedure the following data are collected:

- region of the body interested by fluoroscopy and radiography
- catheters, contrast media and accessories used
- technical radiological parameters: number of films, film sizes, number of sequences, n. of exposures/sequence, fluoroscopic and cine exposure time and mean kV and mA
- number and qualification of staff involved in the x-ray room.

B) A second part more general concerns the activity of the department.
Data on number and type of procedures performed in the last years, type and characteristics of x-ray equipment installed and number and qualification of the personnel are requested.

In the reported period a dosimetric procedure for the evaluation of patient and staff doses during the interventional procedure was developed.

- Staff dose evaluation.
  Each operator will uses two thermoluminescent dosemeters (TLD), one attached over the apron at collar level and the other on the most exposed hand; the TLDs will be used for each patient procedure for the evaluation of staff dose per procedure type in terms of Effective dose, skin dose at the level of the hands and thyroid and crystalline lens doses.

- Patient dose evaluation.
  Due to the variability of direction, position and field size of the beams and the intense use of fluoroscopy during the procedure, Dose Area Product is the dosimetric quantity suitable for patient dose evaluation.
  From information on parts of the body irradiated and directions of the beam, organ doses will be derived per each procedure using appropriate conversion factors derived from MC simulations on mathematical anthropomorphic phantoms. TLDs are also used for the dose evaluation at some organs and tissues (e.g. crystalline lens, thyroid, breast and testes).

The dosimetric system was tested in Neuroradiological department of Udine hospital for the evaluation of patient dose from Chemonucleolysis and Nucleoaspiration interventional procedures. Testes and breast dose were evaluated for 15 patients with TLD measurements while other organ doses were derived from DAP measurements. The following table summarise the results obtained.

Interventional procedure	**Fluoroscopy time (min)**	**Breast dose (mGy)**	**Testes dose (mGy)**	**Effective dose (mGy)**
Chemonucleolysis	3.5	0.7	0.4	0.3
Nucleoaspiration	8.7	0.2	0.2	0.6

Progress Report

Contract: FI3P-CT920013b Sector: C23

Title: Evaluation and management of post-accident situations. Project 1: Database and decision-aiding techniques.

1)	Després	CEA - FAR
2)	Alonso	Univ. Madrid - Politécnica
3)	French	Univ. Leeds
4)	Vanderpooten	Univ. Paris IX

I. Summary of Project Global Objectives and Achievements

The project is devided in two sections :

Section 1 : The EUROGRID data base
The global objective of this part of the contract is to collect and organize data allowing the assessment of individual and collective doses, and of economical impacts of an accidental release of radionuclides at the European scale. This is a continuing task, new data (quantities of collected milk, sheltering factors due to housing) being introduced in the base and old ones (populations, agricultural productions, livestock and employments in different branches of activity) being updated. The current data base, limited to EC countries, will be extended to bordering countries (Switzerland, Austria and Scandinavian countries).
The EUROGRID data base is a part of the data used in the COSYMA Accident Consequence Assessement code that is developed by KfK and NRPB.

Section 2 : Decision aiding system
During the ten last years, interest in the management of post-accidental situations has increased dramatically. Decision making revealed to be a crucial point, so decision aiding methods have been proposed. The objective of this part of the contract is to collect the expertise and to elaborate a decision aiding tool to assist decision makers in the specific field of the management of contaminated foodstuffs. During the development phase, objective parameters (doses and costs) and more subjective parameters (social, political or psycological) are treated by different teams, these two approaches being merged together in a final step.
The goal of the study is to achieve the DACFOOD system, which deals with the objective parameters by developing a cost-effectiveness analysis of the countermeasures that can be envisaged, and to investigate methods allowing to account for expert jugements (subjective parameters).

Head of project 1: Dr. Després

II. Objectives for the reporting period

The Eurogrid data base
The objectives for the reported period were to update the current data files and to extend this data base to other countries.

The DACFOOD decision aiding system
The objectives were to improve the first version of the DACFOOD software and to facilitate the use of this system by external users by preparing a version written in C language. In connection with the associated contracting parties of this contract, the system was used to prepare the scenario of an exercise comparing different aiding techniques methods.

Objectives of the next reporting period

The Eurogrid data base will be developped by adding other parameters, namely the quantities of collected milk and parameters allowing the assessement of doses due to external exposure (sheltering factors as a function of the nature of building materials and living habits).

The DACFOOD system will be tested by potential users and introduced in a larger decision aiding system, including non objective parameters (experts judgment).

III. Progress achieved including publications

The Eurogrid data base
Method used is different according to the parameter : for populations, data are obtained by coupling national census data bases and geographical coordinates of each local administrative areas (municipalities). For other parameters (land use and agricultural productions), data are not available at this level of detail in all the European countries. Moreover, other considerations, as the notion of statistical secret are to be taken into account and limit the degree of accuracy of the basic data. For these reasons, most of the data are issued from the Eurostat publications.
Population files have been updated both at large meshes level (10 000 km^2) and fine meshes level (100 km^2). Populations of Scandinavian countries and Switzerland have been added.
Collection of basic data concerning the other parameters (land uses, agricultural production, livestock, and number of employments in each sector of activity) is in progress, including new parameters (quantities of collected milk, sheltering factors due to housing).

Data from Spain and Portugal are excluded from this work (This part of the study is covered by an other participant).

The DACFOOD decision aiding system

Works have been developped in two ways :

1 - Improvement of the DACFOOD system

The former version of DACFOOD used the GOLDWORKS development tool. Because this system does not allows to distribute run-time version and because maintenance is no longer assured, it has been decided to develop a new version using the C language. In the same time, several improvements have been carried on :

- Dynamic transfer models have been introduced for the different foodstuffs : For milk, it is supposed that the activity increases linearly during the three first days after deposition. In the following days, concentrations vary according to exponential functions. For vegetables and fruits, input data can be the mass activity if deposition occurs at the time of harvest, or surface activity if the vegetable is not at the maturity stage. In this case, different transfert factors are used according to the stage of maturity. To this end, the growing period is devided in four phases : the sowing stage, the beginning of growing, after formation of the vegetable and the time of harvest. It is also supposed that leafy vegetables are a permanent production and that the four stages of growing exists at the same time. For livestock, input data can be the deposited activity, the mass activity of fodder or the meat activity.

- Collective dose assessements are easier due to the availability of needed parameters (populations and agricultural productions) : These data can directly be obtained around the European nuclear sites from the Eurogrid data base.

- The user can automatically generate strategies (a strategy being defined as a set of protective actions simultaneously applicable to different foodstuffs).

This version of DACFOOD is now available, with the user's guide.

2 -Definition of an accident scenario for comparison of different decision aiding techniques

This scenario consists in a release of iodine and caesium isotopes. The site is devided in three areas, according to the amount of deposited material. Each area is defined in terms of population (number of adults, children and infants), agricultural and animal productions and dietary habits (consumption rates and origine of foodstuffs). Consequences of ingestion of contaminated foodstuffs are evaluated (individual and collective dose for each group of population). The compliance of doses and concentrations in foodstuffs with international reglementations is checked, and a diagnostic is given on the need for the implementation of countermeasures. The expected reduction of dose due to the implementation of protective actions, considered one by one, and the cost of each are assessed. Several strategies are defined by the user (or selected

among the strategies automatically generated by the system) and the impact of each in terms of cost and dose reduction is calculated.

Results of these calculations have been transmitted to the others participants to introduce other parameters (as social, political or psychological), for comparison of different decision aiding techniques.

Head of project 2: Prof. Alonso

II. Objectives for the reporting period (September '92 - August '93)

- **To collect data from Spain and Portugal for the updating of the EUROGRID database**, based on the most recent surveys available in national statistics offices.

- **To prepare adequate procedures and programs for the distribution of collected data in the European Grid**, both in the large (10000 km^2) and the fine (100 km^2) meshes.

Objectives for the next reporting period. (September '93 - May '94)

- Processing of the collected data from Spain and Portugal, and transfer of them to IPSN for their incorporation in the EUROGRID database.

- Documentation of the procedures followed for the processing of data.

- Study of the availability of parameters concerning housing and living habits, to be used for the assessment of doses due to inhalation and external exposure.

III. Progress achieved including publications

The EUROGRID database is to be updated by the UPM with data of the Iberic Peninsula (Spain and Portugal) and the Balearic Islands. The data to be transferred to IPSN are referred to land use, cultivated areas, vegetal production, number of livestock, livestock production including specifically milk and milk products, and total employment by economic branches. When possible, these data will be distributed in both levels of the European Grid (meshes of 10000 km^2 and 100 km^2).

During the reporting period, the work has been concentrated in investigating and collecting the available information in the national statistics offices of both Spain and Portugal.

The basic data of Spain have been already acquired and procedures have been prepared for processing the collected data, in order to aggregate them in the categories considered in the EUROGRID database and distribute them in both levels of the European Grid.

Consultations with the *Instituto Nacional de Estatística* of Portugal have allow determining the suitable information, and requesting it in order to start its processing in the next period of the project.

SPAIN:

The following information has already been obtained:

- From the *Instituto Nacional de Estadística*, a magnetic file containing the results of the last Agricultural and Livestock Census (1989), for all the municipalities, approximately 8,000 across peninsular Spain and Balearic Islands. The file, with almost 500,000 records, includes detailed information on land use, cultivated areas and number of livestock.

- From the *Instituto Geográfico Nacional*, a magnetic file with the total surface and the geographical coordinates of the town hall of each municipality.

- The Agricultural and Livestock Yearbook (1989) published by the *Ministerio de Agricultura, Pesca y Alimentación*, from which the crops and livestock yields are being taken, so that combined with the above indicated Census the vegetal and livestock productions could be distributed in the European Grid.

- The Regional Accounting of Spain (1986-1989, base 1985), both in book and magnetic media, published by the *Instituto Nacional de Estadística*, including the distribution of the number of employments by economic branches for each autonomous region of Spain (NUTS II). And the publication *"Renta Nacional de España y su distribución provincial"* by the private *Bank of Bilbao-Vizcaya* which contains estimated number of employments by economic branches in each province (NUTS III), that could be used combined with the above for the distribution of these data in the European Grid.

Several programs have been elaborated to allow for the distribution of the different kind of data in both levels (10000 km^2 and 100 km^2) of the European Grid:

- Those concerning land use and cultivated areas are working with the fractions of each type of land or crops in each municipality, which are assigned to the grid element in which the town hall of the municipality is located. For grid elements not containing any town, the corresponding fractions to the closest and largest municipality are being used.

- For the crop productions, the cultivated areas are multiplied by the average yield corresponding to the province in which the municipality is located.

- For the number of livestock, the figures reflected in the Census are assigned to the location of the municipalities in the grid. Milk and milk products are obtained using the average yield corresponding to the province in which the municipality is located.

- For the number of employments, average fractions of total employment per inhabitant for each economic branch, in the autonomous region to which the municipality belongs, are applied to the total population of each municipality, and then distributed in both levels of the European Grid.

PORTUGAL:

Diverse contacts with the *Instituto Nacional de Estatística* have finished with the recent purchasing of the following information:

- Results in book and magnetic media of the General Agricultural and Livestock Census (1989) for the 66 agricultural zones in which Portugal is subdivided. They include land use, cultivated areas and number of livestock.

- Agricultural Yearbook (1989) including the average yields for crops and livestock products to be used, combined with the results of the Census, for the distribution of vegetal and livestock productions.

- Distribution of employment by regions (NUTS II) and economic branches (1989).

Similar procedures to those used in the case of the Spanish data will be prepared in order to complete the integration of all these data in the EUROGRID database.

Head of project 3: Dr French

II. Objectives for the reporting period

1. Input on the use of multi-attribute value/utility methods for comparison with the outranking/electre methods used by Professor Roy.

2. The development of decision conferencing approach to use in these circumstances.

3. Sensitivity analysis and robustness studies.

III. Progress achieved including publications

A version of multi-attribute value analysis software which will integrate with the RODOS DSS is being developed and is in beta test. This will enable multi-attribute analysis and a visual sensitivity analysis to be conducted within the RODOS environment and will enable RODOS to support a decision conference post accident. It is intended to incorporate some of the sensitivity analysis code written at Leeds using the methodology developed by French and Rios Insua[1].

Initial comparisons of the outranking and multi-attribute value/utility approaches have begun.

A set of notes on the use of multi-attribute value and utility analysis aimed specifically at the radiation protection community is in preparation.

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[1] S.French and D. Rios Insua (1991) 'A framework for sensitivity analysis in multi-objective decision making' *Eur. J. Opl. Res.* **54**, 176-190.

Related work:

A decision conference based upon simulated post accident data was run in Denmark in December 1992 under the auspices of the Nordic Cooperation Programme BER-3 group[2]. The data and experience gained will be available to the current project.

[2] S. French, O. Walmod-Larsen and K. Sinkko (1993) 'Decision conferencing on countermeasures after large nuclear accident' *Riso-R-676(EN)*, Riso National Laboratories, Roskilde, Denmark.

Objectives for the reporting period

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Head of project 4: Mr Vanderpooten

II. Objectives for the reporting period

The general purpose of our work is to determine the potential benefits of a multiple criteria methodology for supporting decisions concerning the evaluation of countermeasures in post accidental situations.

A first objective was to review the scientific literature dealing with the use of decision aiding techniques in radiation protection.

The main objective was then to develop a new multiple criteria methodology, taking into account the limitations which had been noticed when reviewing the literature. We decided with the other members of the group to test such a methodology on the specific context of the management of contaminated foodstuffs. More precisely, the tools to be proposed should support the selection of countermeasures.

III. Progress achieved including publications

Concerning our first objective (review of the scientific literature), it should be noticed that the number of applications of decision aiding techniques to radio protection is quite limited (particularly, if we consider multiple criteria approaches). An interesting feature of some reported applications is the use of a decision conference environment. Such decision conferences appear to be useful when aiming to support negotiation and decision among several decision makers.

However, the major limitation we observed in most of the reported applications is that the techniques used only deal with a very limited number of alternatives. In our case, the number of alternatives (countermeasures) is potentially very large. Indeed, countermeasures result from the combination of elementary countermeasures (concerning different areas, different types of foodstuffs, different decontaminating processes).

This review of the literature and a better knowledge of the problem of the management of contaminated foodstuffs allowed us to establish the following requirements regarding the decision aiding technique to be developped:

- it must be able to handle **large sets of alternatives;**
- it must explicitly involve **multiple criteria;**
- it must consist of a **flexible** tool allowing to support **negotiation** among different actors.

Such a method could then be easily integrated in a decision conference environment.

Notice that these requirements are in accordance with the objectives and the experience of the other participants to the project. Indeed, Simon French (Leeds University) already experimented decision conferences and advocates its use in such a context. Jean Brenot, Alain Despres and their team (IPSN) developped a tool (Dacfood) which allows to evaluate countermeasures in terms of costs and efficiency.

More precisely, the proposed methodology consists of two major stages:

1. generation of a **representative** sample of countermeasures
2. elaboration of a multiple criteria decision aiding method supporting an **evolutive exploration** of the sample of countermeasures

Stage 1:
As indicated before, the number of countermeasures is potentially very large since it results from the combination of elementary countermeasures. Many of these combinations are either unfeasible or clearly without interest. The number of remaining combinations may still be rather large (several thousands in a typical application). We suggested to incorporate expert knowledge so as to reduce the set of combinations and get a representative sample of countermeasures (at most one or two thousands). Each countermeasure present in the sample would be evaluated according to several criteria measuring its impact. In a first step, the criteria to be considered are cost and efficiency (in terms of residual doses after application of the countermeasure). These evaluations can be obtained from Dacfood. In a further step, the possibility of integrating other criteria could be considered (e.g., psychological or political impact, using a qualitative scale).
Notice that this stage would be performed prior to the decision conference.

Stage 2:
This stage concerns the method to be used during the decision conference. This method should allow the decision makers to explore the countermeasures generated at stage 1. The method we are proposing is a multiple criteria interactive procedure. It is designed so as to allow the decision makers to express their requirements regarding the countermeasure to be taken. Then, the procedure looks for the available countermeasure which best fits their requirements. The resulting countermeasure is presented to the decision makers who can react by expressing additional or new requirements. This iterative process can be repeated until some satisfactory countermeasures are obtained. The procedure is flexible since it allows changes of mind and explorations in a trial and error fashion.

An experimental prototype of this procedure is currently being developped. We are planning to test this procedure on a decision exercice.

Progress Report

Contract: **FI3P-CT930068** **Sector: C23**

Title: Assessment and management of post accidental situations . Radiation detriment, risk perception and risk communication.

1) Brenot CEA-FAR
2) Joussen IFS

I. Summary of Project. Global objectives and Achievements

Risk perception and communication studies are essential for responsible risk management in that they provide : a) a basis for assessing the likely response of workers and the public to advice and information on radiation protection which is important for both normal (operating) conditions and abnormal situations; b) a route to appraising the effectiveness/efficiency of radiation protection; c) criteria and guidelines for coping with problems of risk attenuation and risk amplification.

The research proposed has two main objectives :
(1) to undertake research in order to improve the understanding of risk perception in relation to radiation detriment ; and
(2) to evaluate current approaches in risk communication, and to develop appropriate strategies for radiation risk communication in the European Community (EC).

Four tasks are developed :
Task 1.Review of risk perception studies
Task 2.Review of evaluation techniques for risk communication programmes
Task 3.Assessing organisational factors influencing social learning in radiation protection
Task 4.Identification of strategies for coping with risk amplification and attenuation problems

Two teams are involved : CEA-FAR (IPSN, FRANCE), Head of project : Dr. BRENOT, and IFS (RWTH-AACHEN, GERMANY), Head of project : Dr. JOUSSEN. CEA-FAR will develop Task 1 when IFS develops Task 2 . Both teams are concerned by Tasks 3 and 4.

Head of project 1: Dr. BRENOT

II. Objectives for the reporting period

The review of risk perception studies constitutes the first step of the proposed research. Its objectives are firstly, to evaluate the concepts of risk perception, secondly to assess their quantification in view of improving radiation risk communication, and finally to generate an appropriate reference framework for developing communication strategies.

Risk perception associated with any hazard (situation, technology, or product) depends on the social representation of the hazard, especially when the hazard is not experienced bodily by individuals during their everyday life, e.g., as with nuclear power. Is has been observed in general that each hazard has not only one social representation but many, and this plurality of representations is due to many factors, some related to the person (e.g.,demographics, experience and skills, personality, social status, cultural level, as well as political positions), others related to the risk source (e.g., economics, institutional and legal constraints).

The first requirement is to clarify the social representations of radiation risk and the factors which may contribute to the amplification or attenuation of radiation risk perception.

A second point is the changeability of a representation which occurs mainly after particular events (such as catastrophes, political elections, or communication campaigns). Case studies, e.g., on Chernobyl or on Sellafield, could be used to enlight the point.

Furthermore, the debate about hazard issues can be better understood when embedding it in the context of other social debates.

It is also needed a clear understanding of the various individual coping strategies. These individual behaviours range from apathy, no worry, avoidance, information seeking, changes in life style, inter alia. How they occur and when is necessary information for the development of better risk communication programmes.

III. Progress achieved including publications

A major effort has already been made during the previous period July 1985 - May 1992 within the Contract B16-122-F(D) devoted to the "Assessment of the subjective dimension of

the radiological detriment, in relation to sociological considerations". The document on risk perception which was prepared for the last four sessions of UNSCEAR will serve as a basis for this review of risk perception studies.

Areas to be extended concern risk targets (i.e., risk to you and your family and risk to society as a whole or in other words, personal risk vs societal risk), social representations (definition and plurality), cultural theory of risk (definition, relation with life styles, use in the three countries USA, Sweden and France with a cross-national comparison), and to end a more detailed description of attitudes and behaviours.

Two surveys have been realized which give French data on the two topics of risk targets and cultural theory. The first survey was made in March 1993 in association with social-psychologists of Paris University René Descartes. It was a pre-test for preparing the questionnaire used in cultural theory. The second survey was a national one realized by IPSN in May 1993 where the questionnaire was included in parallel with some dimensions of risk perception and particularly those of personal and societal impacts. Basic results of the two surveys are given in [1] and [2] respectively.

Detailed analysis of the national data is in progress. It will be included in the risk perception review.

[1] BAUZADAT Ph. Perception des risques. A la recherche des facteurs socio-culturels. Mémoire de stage. Université Paris V René Descartes, Septembre 1993.
[2] BONNEFOUS S. et J. BRENOT. Perception des risques et de la sécurité : résultats du sondage de Mai 1993. Note SEGR/LSEES 93/20, Juin 1993.

Head of project 2: Dr. JOUSSEN

II. Objectives for the reporting period

The first objective was the development of a radiation / risk matrix in order to identify critical areas where the public concern is related to radiation hazards. Besides the, nearly traditional, risk-awareness in the nuclear energy/waste area we wanted to put emphasis on radiation risks in alternative areas like in medicine or the everyday life. Considering the communication processes in different fields our aim was to show that risks are much less stretched in areas like medicine since traditional associations going along with health care or medical treatment are mainly positively connotated.

In a next step our goal was to fix the possible risks with event/application and the question how people perceive and to what extent they discuss any presumed risks. We assumed that awareness of risks going along the use of nuclear power is supposingly more established in the common mind than recently discussed risks like using X-rays or in food preservation. In order to find an appropriate approach to these phenomena we were supposed to look for paradigms that, in contrary to traditional risk analysis, underlined more the social and political factors as significantly influencing the perception of risks. Taking into account the differences that can be drawn from international comparisons in risk perception (for exemple concerning radon awareness) we also aimed at searching for cultural approaches that may offer insight into public perception processes from an intercultural perspective.

In the near future the goal of our research will be the identification of risk communication programs in these different fields and subsequently the investigation of the efficiency of varying communication strategies. We have chosen interviews as the main methodological instrument in order to get access to different modes of risk perception ; additionally we expect to achieve information about successful / failed communication approaches that will help to develop criterias for effective risk communication.

III. Progress achieved including publications

How can we as a society tackle the problems of radiation hazards when there is insufficient knowledge about the public concerns and uncertainty about the risks even from the experts' point of view ? In order to deal with this problem the first stage of our research design aimed

at getting a better understanding of how radiation is perceived by the public in different fields of application and to what extent experts consider these effects of radiation as hazardous.

We intended to identify the main fields where risks generated by radiation affect society, mainly by scanning the media, and to browse recent litterature dealing with nuclear issues and related fieds like using X-rays in medicine, food preservation, or cosmic rays. Having become insight into the sensitive areas our conclusion was to mainly focus on the fields nuclear technology (accident + wastes), medicine (X-rays, radiodiagnostic, radiotherapy), industry (food preservation) and everyday life (TV, cosmic rays).

Taking into account the broad scope where radiation may create or already has created public debates we decided that talking about radiation risks has to start from the most prominent concern people refer to, is to say radiation generated by nuclear power plants (normal operation and accidents) / nuclear weapons, and subsequently nuclear waste. In contradiction to these concerns in the nuclear sector the risks of radiation in other fields, particularly that of medicine, often seem to be neglected or underestimated. After having identified the fields where radioactive radiation may affect the health of the public and having compared hazards with their actually perceived risks the goal of our next step consisted of the evaluation of how the public communicated about the particularly risks and to what extent the availability of information changed attitudes towards these risks.

Studies dealing with nuclear hazards and those concerned with chemicals or other technologies came to similar conclusions : they all underline how individual, social and cultural differences have significant influence on risk perception and risk communication in modern societies. Informing about risks has to take into account the social and cultural environment the information will be embedded in. Disagreement mostly is not a question of insufficient data but different interpretation of data and subsequently varying conclusions. Traditional approaches in risk communication lacked a profound understanding of the context in which risks were perceived and either amplified or attenuated. Nonetheless the heavy controversies in this field contributed to a better understanding of these processes, and introduction of new technologies may learn from experiences gained during those discussions.

One of the most important issues in the fields of nuclear technology however is related with the fact that corresponding risks are not voluntarily taken by individuals. Buying a TV set or choosing the profession pilot is an individual decision, and only the user puts himself at risk ; in the same way controversies about X-ray application in medical treatment may easily be settled by communicating with the physician. However the problem in these areas still is insufficient knowledge about possible hazards going along with new products or processes and uncertainty creates speculation and irrational fears. This is due to the differing statements

of experts/institutions about presumed risks that cannot be figured out yet with convincing scientific evidence.

According to our research design we will undertake interviews in the next future. The main objectives are the identification of risk management approaches using risk communication as a tool to deal with social representations of radiation risks. We will continue as well to investigate different organizational arrangements that try to comply with the necessity of organizational learning in order to deal with radiation hazards.

As a result of the ongoing research a publication on risk communication in the field of radiation is forthcoming at the end of 1993.

Progress Report

Contract: **FI3P-CT920023** **Sector: C24**

Title: CEC/USNRC joint project on uncertainty analysis of probabilistic accident consequence codes.

1)	Goossens	Univ. Delft
2)	Haywood	NRPB
3)	Ehrhardt	KfK
4)	Boardman	UKAEA
5)	Roelofsen	ECN
6)	Hofer	GRS

I. Summary of Project Global Objectives and Achievements

The objectives of the proposed joint project are to further develop and apply expert judgement elicitation techniques in estimating the uncertainties associated with the predictions of probabilistic accident consequence assessment codes, and to investigate the use of the results of these studies as input to uncertainty analyses of such codes.

Within the project the following contributions will be made:

(i) To further develop methodological backgrounds and implementational aspects of expert judgement elicitation techniques to be applied in the joint project (TUD contribution).

(ii) To further develop mathematical techniques for handling modelling uncertainty (TUD contribution).

(iii) To perform modifications and extensions of the System for Uncertainty and Sensitivity Analysis (SUSA), that emerge from methodological improvements, for instance from (ii), as well as from the practical applications of the system to results from the COSYMA and possibly MACCS codes (GRS contribution).

(iv) To provide the necessary detailed information on the relevant parameters and models, in order to:
   a. assist in the selection of appropriate experts in specific subject areas of consequence assessment (NRPB-KfK-SRD-ECN contributions),
   b. provide experts both in the general area of accident consequence assessments, and in particular modelling aspects, who will interact with and advise the chosen experts in other fields (NRPB-KfK-SRD-ECN contributions),
   c. consider the implications of the results of the expert elicitation with regard to an uncertainty analysis of the COSYMA code (NRPB-KfK contributions).

It has been decided to start off (November 1992 - November 1993) with the following subject area:

- Atmospheric dispersion and deposition (KfK-NRPB).

The other areas are further investigated in order to prepare directions for choices of the most relevant parameters (NRPB-KfK-SRD-ECN).

Head of project 1: Dr. Goossens

II. Objectives for the reporting period

Within the joint project the chosen expert judgement technique is a combination of the techniques prior developed at TUD and applied in the CEC pilot study on dispersion and deposition (report 91-81 by Roger Cooke, TUDelft), and the methods previously used by the U.S. Nuclear Regulatory Commission (published under NUREG-1150). The objectives for the reporting period are

1. to apply the expert judgement technique to the area of atmospheric dispersion and deposition
2. to further develop post-processing techniques applicable for the chosen area.

III. Progress achieved including publications

Major achievements for the areas of dispersion and deposition were:

- nominations of 8 European experts (4 for dispersion and 4 for deposition)
- formulation of elicitation variables
- formulation of seed variables for performance based weighting schemes
- further development of post-processing techniques (the software packages PARFUM and CORRUPT)
- joint execution of dry run exercise (March 1993)
- support of training session (April 1993)
- elicitation of experts (May 1993)
- processing of combined assessments from individual expert assessments
- post-processing of the uncertainties of the combined assessments into uncertainties of the COSYMA-code parameters for dispersion and deposition

Publications:

F.T. Harper, L.H.J. Goossens e.a.
Project Plan for Joint CEC/USNRC Model Probability Elicitation
for CEC DG XII/Brussels, CEC DG XI/Luxembourg and USNRC/Washington, DC, USA. Sandia National Laboratories, Albuquerque, NM, USA/TUDelft, Safety Science Group, December 1992

L.H.J. Goossens, F.T. Harper, R.M. Cooke, S.C. Hora, G.N. Kelly and J.C. Glynn
Uncertainty analysis of probabilistic accident consequence codes for the nuclear industries - A joint CEC/USNRC expert judgement study
Invited contribution (paper) to the International Conference on Mathematical Models and Supercomputing in Nuclear Applications, 19-23 April 1993, Karlsruhe, Germany

Head of project 2: Miss Haywood

II. Objectives for the reporting period

The NRPB objectives for this period were

(i) to assist in the selection of appropriate experts in specific areas of consequence assessment,

(ii) to provide both in the general area of consequence assessments and in particular modelling areas to interact with and advise the chosen experts in other fields. NRPB is leading in the areas of food chains, internal dosimetry and late health effects.

III. Progress achieved including publications

NRPB organised and hosted a meeting of CEC contractors to discuss the division of the whole consequence field between the different panels established. This enabled possible overlaps between some of the panels to be resolved.

The main part of the work of the project as a whole has been in the dispersion and deposition area. NRPB is to assist KfK in this area. Suggested experts in the area were identified. The variables whose uncertainty may make a major contribution to the overall uncertainty have been agreed with KfK. These are the variables for which distributions should be obtained using expert judgement. The questions to be used in obtaining ranges from the experts were also agreed with KfK.

NRPB is taking the lead in determining the variables and elicitation questions for the food chain, late health effects and internal dosimetry sections of the analysis. Discussion notes identifying the important parameters for these aspects of the analysis have been prepared, and sent to Delft for discussion with USNRC. The major part of the NRPB work in this contract will come when the expert panels for these topics meet.

Head of project 3: Dr. Ehrhardt

II. Objectives for the reporting period

Based on the experience gained with uncertainty and sensitivity analyses of the former accident consequence code UFOMOD, KfK will provide input to the expert judgement elicitation project and the subsequent uncertainty analyses with COSYMA. Main objectives of the reporting period were to contribute to the first panel on atmospheric dispersion and deposition with support of NRPB

- in assisting to select appropriate experts for dispersion and deposition processes,
- in identifying the relevant model parameters used in the elicitation procedure,
- in performing the expert judgement elicitation exercise.

Objectives for the next reporting period:
KfK will contribute to the preparation of the next panels in assisting in the selection of appropriate experts and in selecting the relevant models and seed variables. Two subject areas are envisaged:

- early health effects (in support of ECN)
- food chain modelling (in support of NRPB).

III. Progress achieved including publications

During several meetings advice was given to the planning staff of the uncertainty project concerning phenomena of the atmospheric boundary layer and measuring and modelling of atmospheric dispersion and deposition. Especially the following items were treated:

- the dispersion and deposition modelling in the COSYMA code;
- the choice of the following elicitation and seed variables:
  - χ_C/Q normalized centreline concentration
  - χ_Y/χ_C relative concentration at a crosswind location
  - χ_Z/χ_C relative concentration above centreline
  - σ_Y standard deviation of crosswind locations at release height

  and for some cases
  - σ_Z vertical diffusion parameter
  - χ_G/Q normalized ground level concentration
  - v_D dry deposition velocities for a number of surface types and aerosol particle sizes
  - f_W fraction of material removed by wet deposition (different materials, rain durations and rain intensities);

- the unequivocal formulation of the elicitation questions, i.e. the definition of the meteorological situation as it is needed for the input of a Gaussian model under consideration of typical uncertainties;
- definition of the sources of uncertainties to be taken into account, i.e. restrictions in the complexity of the dispersion and deposition scenarios (no unstationary meteorological conditions, no mountainous terrain), and which sort of uncertainty should be considered by the experts and which not.

The experts were introduced into the following subjects by oral presentations:
1. modelling of atmospheric dispersion and deposition in the COSYMA code
2. types of uncertainties to be considered by the experts and restrictions in the complexity of the meteorological situations.

There was a discussion about the general validity of the results of the uncertainty study concerning the limited complexity of the scenarios chosen. In connection with this, a study considering German reactor sites and their weather statistics and deriving from this the frequencies of 'Gaussian' and complex dispersion situations was presented.

Furtheron, concerning the expert elicitation procedure, support was given in identifying experts in Germany, in performing the 'dry run' acting as a test expert, and in the elicitation of four experts and the preparation of the elicitation protocols.

Head of project 4: Miss Boardman

II. Objectives for the reporting period

- To participate in the European Project Committee Meetings and to peer review the plans for the initial phases of the project.

- To finalise the content of the expert elicitation panel for which UKAEA is responsible, namely the 'Behaviour of Deposited Material and Calculation of Related Doses'.

III. Progress achieved including publications

During the first phase of the CEC/USNRC joint exercise, UKAEA provided a peer review role for all activities on the project. They were present at all European Project Committee meetings and actively participated in the discussions. In February 1993, the scope of the panel 'Behaviour of Deposited Material and Related Doses' was produced by UKAEA for the project committee, following discussions held at the last project meeting at NRPB in January 1993.

Since that meeting, UKAEA has begun to review the literature covering the subject areas within their expert panel, but will limit the activities to a minimum until a final decision to proceed with the panel is received from the CEC. This decision will hopefully be made by the end of 1993.

Head of project 5: Ir. Roelofsen

II. Objectives for the reporting period

As mentioned in item I the subject area for the reporting period was 'atmospheric dispersion and deposition'. Because ECN's activities will be in the areas of 'early health effects', 'late health effects' and 'behaviour of deposited material' no substantial activities were foreseen for the reporting period.

III. Progress achieved including publications

In January 1993 ECN attended the informative meeting at NRPB, U.K. Besides some administrative work, no other activities were performed in the reporting period.

Head of project 6: Dr. Hofer

II. Objectives for the reporting period

1. Assist in establishing the link between SUSA and the accident consequence assessment code (or module) subject to analysis.

2. Perform modifications and extensions of SUSA that emerge from the actual application of the system to results from the COSYMA code.

III. Progress achieved including publications

Ad 1.
Model runs may now be performed on a workstation under the control of SUSA-PC using NFS to link Personal Computer and workstation.

Ad 2.

- Documentation of the analysis input if offered as a compilation over all parameters and parameterwise in the form of single data sheets
- Inclusion of an option to request model runs with best-estimate or reference values of the uncertain parameters
- Extensions to the set of supported distributions
- Upgrade to MS-WINDOWS 3.1 and MS-EXCEL 4.0
- Update of the User's Guide
- Continued testing of and corrections to SUSA-PC.

Publication:
M. Kloos
SUSA-PC: A Personal Computer Version of the Program System for Uncertainty and Sensitivity Analysis of Results from Computer Models, Version 1.0, USER'S GUIDE Gesellschaft für Anlagen- und Reaktorsicherheit, Garching, July 1993.

Progress Report

Contract: FI3P-CT920036 Sector: C24

Title: Development of a comprehensive decision support system for nuclear emergencies in Europe following an accidental release to atmosphere.

1)	Ehrhardt	KFK
2)	Gland	EDF
3)	Müller	GSF
4)	French	Univ. Leeds
5)	Sohier	CEN/SCK Mol
6)	Haywood	NRPB
7)	Bleasdale	Nuclear Electric

I. Summary of Project Global Objectives and Achievements

The main aim of the project is the development of RODOS, an integrated and comprehensive real-time on-line decision support system for nuclear emergencies in Europe. The system will be able to make consistent predictions from the vicinity of the release in the early phase of an accident to far distant areas and at later stages unperturbed by national boundaries. It will integrate methods, models and data which allow the continuously updated estimation of the present and future distribution of activity concentrations in the environment, which offer the possibility to simulate different intervention strategies for all kinds of countermeasures in order to assess in advance their respective merits and disadvantages in terms of dose or health effects saved and the associated social and economic costs, and which enable intervention strategies to be ranked in terms of their effectiveness, practicability and acceptance.

Within the first phase of the project, the overall structure and the hardware and software framework of RODOS have been developed as a transportable package, which supports the integration of external programs provided by the contractors. The first prototype version RODOS-PRTY 1.0 has been realised in autumn 1992 and presented internationally at several conferences to the radiation protection community. It already showed its basic functions and graphical output, however still limited in its spacial and temporal applicability.

Main objective of the present contract period is to further develop the software framework, to improve and extent the programs and data bases provided by the contractors, and to integrate the software in the version RODOS-PRTY 2.0 scheduled for mid 1995. This version will show the full functionality of the design specifications and will serve as the basis for the first pilot-version for operational use.

Progress has been made within the reporting period in the

- development of data assimilation methods incorporating both monitoring data and model predictions for obtaining consistent pictures of the environmental contamination and the source term (Univ. of Leeds, SCK/CEN Mol),

- improvement and extension of the modules ATSTEP-CORA (atmospheric dispersion and deposition; KfK), EMERSIM (simulation of emergency actions; KfK), ECOAMOR (exposure pathways and dose calculation;GSF), and FRODO (simulation of relocation and agricultural countermeasures; NRPB),

- preparation of training courses using RODOS as illustrative tool (NE, EdF, KFK),

- extension of the functions of the RODOS operating system OSY, in particular of RoGIS, its geographical information system (KfK).

Head of project 1: Dr. J. Ehrhardt

II Objectives for the reporting period

The overall project objective is the further development of RODOS-PRTY 1.0 to an operational flexible system applicable in West and East European countries. A second prototype version with full functionality is scheduled for mid 1995. To achieve this goal, KfK work is concentrated on the following topics:

(1) further development of the hardware and software framework including the functions of the geographical information system RoGIS;

(2) incorporation of software products and data provided by the contractors, test of their functions, and generation of the appropriate user interfaces, in close cooperation with the developers;

(3) further development of modules and data bases for simulating emergency actions and estimating economic costs and health effects;

(4) on-line connection of the system with radiological and meteorological information networks and processing of data;

(5) preparation of systematic course material together with illustrative scenarios for training and exercises.

III. Objectives for the next reporting period

The goals set out for the reporting period cannot be achieved within one project year. Therefore work on the topics (1) to (5) listed above will continue in the next reporting period with priorities determined by the availability of software and material provided by the other contractors.

IV. Progress achieved including publications

1. System development

Within the framework of the Commission's Radiation Protection Research Action, KfK has prepared in autumn 1992 the first prototype version RODOS-PRTY 1.0; it was demonstrated during international conferences /1,2/ and national radiation protection committee meetings. It reflects the objectives, key features and

structures described in the design study and the hardware and software framework documents broadly agreed by the contractors. In particular, the RODOS operating system OSY has been developed in the programming language C as a portable package to run with a UNIX operation system, and X-Window user interface with OSF/MOTIF extensions. The data management is based on SQL standards.

Important sources of information for setting priorities in development plans will arise from the installation, use and test of RODOS-PRTY 1.0 in cooperating institutes. Ideas for extensions and improvements of models, data bases and endpoints will emerge which will help to progress towards a broadly accepted system for operational use. Therefore, a first training course for future RODOS developers has been organized at KfK from 21 June to 30 June 1993. 9 Participants from institutes involved in the RODOS project (Greece, Poland) and the associated Joint Study Project 1 (JSP1) of the CEC/CIS Joint Programme on the Consequences of the Chernobyl Accident (Russia, Ukraine, Belorus) attended the course. Copies of RODOS-PRTY 1.0 will be transferred and installed in these institutes in the second half of 1993 and the first half of 1994.

Using the experience gained during the RODOS demonstrations and the training course, the following improvements of the software framework (operating system OSY) have been made during the reporting period and partially incorporated in the prototype version PRTY 1.0, ready for release by September 1993:

- implementation of a communication server on the basis of TCP/IP-distributed computing network system for improving the interprocess communication;
- modification of the editors-user-interfaces taking into account comments of the participants at demonstrations;
- discussion, comparison and agreement on the data base management system (INGRES) and the graphics system (PHIGS) for RODOS; investigation of coupling facilities for on-line operation of RODOS;
- development of the design of RoGIS, the geographical information system of RODOS; test and implementation of basic structures;
- preparation of proposals for the layout of and the guidelines for the future RODOS documentation as recommendation for the contractors.

2. External programs

The subsystems ASY (analysing subsystem) and CSY (countermeasure subsystem) of RODOS-PRTY 1.0 contain first versions of the modules ECOAMOR and FRODO

from GSF and NRPB, respectively, and the complete RESY-software from KfK /3/. It comprises the modules ATSTEP-CORA (near-range atmospheric dispersion and deposition), EMERSIM (emergency actions) together with subroutines for calculating doses, HEALTH and ECONOM (health effects and economic costs). The revised structure of ASY and CSY together with the modules for assessing consequences and countermeasures is shown in Figs. 1,2. The PAD, MCF and RIMPUFF software will be developed within the associated contract FI3P-CT92-0044 on meteorology and atmospheric dispersion.

Within the contract period, the following improvements have been made in the modules ATSTEP-CORA and EMERSIM:

- dynamic grid size for selecting arbitrary calculation areas;
- flexible input of spacial and temporal variable rain fields and of the timing of emergency actions;
- test of the "windfield"-option in ATSTEP allowing for dispersion calculations allong forecasted meteorological fields;
- development of the methodology and the structure of the simulation module EVSIM for calculating evacuation routes and times; coding of a first demonstration version.

In parallel to the further development of the RODOS system and the external programs, work at KfK together with EdF, Nuclear Electric and SCK/CEN Mol aimed at the application of RODOS for training and in exercises. During two meetings an agreement was reached on the type, content and working programme of a training course for health physics managers, taylored to the functions and possibilities of RODOS. By the beginning of 1994, the detailed structure and teaching programme of the course including illustrative scenarios and the requirements on the RODOS system (endpoints, graphics) will be finally discussed and fixed.

3. Coordination

At the beginning of 1993, the Commission's Services established the RODOS Management Group (RMG) to assist them in the overall management and coordination of the second phase of the RODOS project. The first meeting of the RMG has been held at KfK on 17 February 1993.

As agreed during this meeting, periodic RODOS newsletters will be issued in three to four month time intervals. They will help to establish an effective communication between all RODOS contractors. The RODOS newsletters will be prepared by

KfK with support of the coordinators of the subprojects and with input from the CEC and the contractors. The first issue was distributed in May 1993.

In order to coordinate work of software developers, several meetings were organized at KfK within the reporting period:

- Further development of ASY, CSY and ESY modules, 10 -12 February 1993 (NRPB, GSF, CEA, KfK);
- System hardware and software structures, 18 Feburary 1993 (ICSTM, Univ. of Leeds, KfK);
- Meteorology and atmospheric dispersion, 24 - 25 February 1993 (Democritos, ENEA, EdF, GRS, RISØ, KfK);
- Coordination meeting with CYFRONET (Poland), 11 - 12 March 1993.

A formal registration procedure for all RODOS documents has been established retroactive from 1 February 1993. The document registration and the issue of document numbers is under the control of the respective coordinators. A copy of any document is held at KfK as the central depository for all RODOS documentations.

Besides the existing close cooperation with the contractors of the projects FI3P-CT92-0044 and FI3P-CT92-0013b, a direct link exists to the Joint Study Project 1 (JSP1) of the CEC/CIS Joint Programme on the Consequences of the Chernobyl accident, in which at present KfK is also acting as coordinator.

Publications

[1] J. Ehrhardt, J. Päsler-Sauer, O. Schüle, G. Benz, M. Rafat, J. Richter
Development of RODOS, a comprehensive decision support system for nuclear emergencies in Europe,
3rd International Workshop on real-time computing of the environmental consequences of an accidental release to atmosphere from nuclear installation,
25 - 30 October 1992, Schloß Elmau, Bavaria, to be published in Radiation Protection Dosimetry

[2] J. Ehrhardt, F. Fischer, J. Päsler-Sauer, O, Schüle, G. Benz, M. Rafat
RODOS and RESY: two integrated real-time on-line decision support systems for nuclear emergencies
Proceeding of the Joint International Conference on Mathematical Methods and Supercomputing in Nuclear Applications M&C + SNA, 19 - 23 April 1993, Karlsruhe, Germany, Vol. I. p. 319 - 330

[3] J. Päsler-Sauer
Assessment and evaluation of early countermeasures and consequences in RODOS/RESY
3rd International Workshop on real-time computing of the environmental consequences of an accidental release to atmosphere from nuclear installation,
25 - 30 October 1992, Schloß Elmau, Bavaria, to be published in Radiation Protection Dosimetry

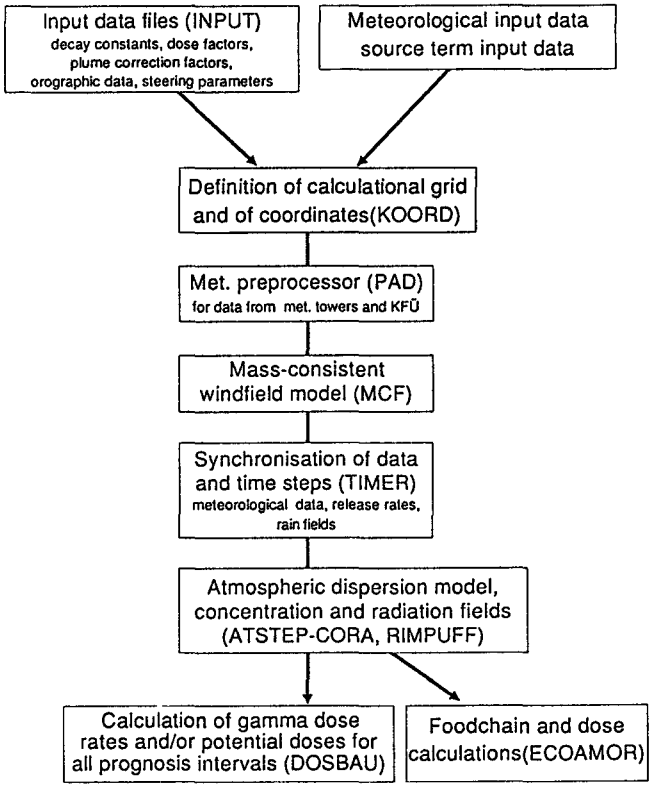

Fig. 1: RODOS-PRTY 1.0: Structure of the analysing subsystem ASY

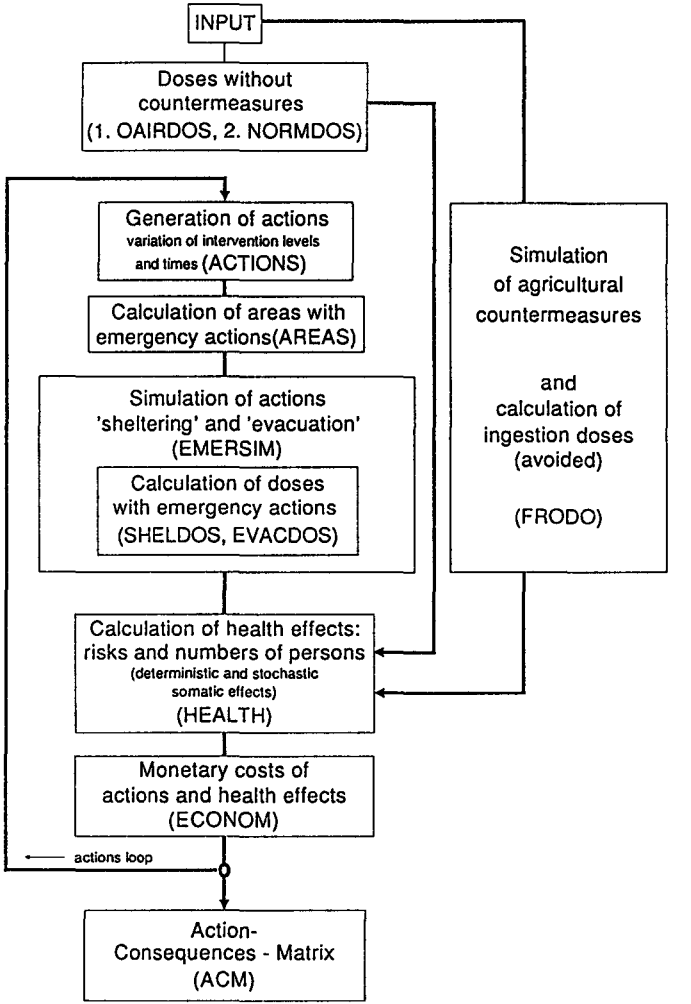

Fig.2: RODOS-PRTY 1.0:Structure of the countermeasure subsystem CSY

Head of project 2: Mr Gland

II. Objectives for the reporting period

EDF, operator of 56 power reactors, has implemented a structure to manage accident situations and has developed models designed to estimate the consequences of radioactive releases.

Within this context, EDF contributes to the development of RODOS by bringing its experience in implementing a crisis structure, in emergency training and excercising and in operating meteorological and atmospheric dispersion models.

III. Progress achieved

Mainly, EdF worked within the subgroup "training". The other participants to this subgroup are KfK, Nuclear Electric and SCK/CEN Mol.

A first meeting was held at KfK in September 1992. According to the decisions taken during this meeting, we gathered informations about the training courses dealing with radiation protection held at EDF. We made a detailed review of three of them.

The first one is a general radiation protection course. It addresses to engineers working at nuclear plant and/or involved in nuclear safety. It covers basic elements such as biological effects of radiation or general safety principles, and more specific ones such as radioactive releases or local and national EDF crisis organization.

The two other courses concern the local EDF crisis organization managers, namely our Health Physics managers and our decision makers.

EDF has organized the second meeting of the subgroup "training" (6-7 April 1993). At the end of this meeting, the participants agreed to develop a specific RODOS training course for Health Physics Managers as described in our own course. Seven course modules have been defined.

EDF will participate in the development of 3 of these modules:

- to outline the basis of potential accident scenarios and source terms (with support of Nuclear Electric),

- to describe the basic effects of meteorology and atmospheric dispersion on contamination patterns (with SCK/CEN Mol as leading institution),
- to develop monitoring strategies (with SCK/CEN Mol and Nuclear Electric).

The objective is to present the draft structure and content of each of these modules during the next meeting at Mol in November 1993.

We also participated at a RODOS coordination meeting on meteorology and atmoperic dispersion (KfK, 24-25 February 1993).

There, we have presented the concept of the ECRAN project. The aim of this research and development project was to provide real time and forecast estimates of the radiological consequences of a release, whatever the scale of the radioactive cloud dispersion. This project has been done between 1987 and 1989 and was presented at the 2nd international workshop on real-time computing of the environmental consequences of an accidental release to the atmopshere from a nuclear installation, Luxembourg, May 16-19, 1989. The concept of this project (meso scale forecast model using the results of the French Met. Office forecast model, local scale model using on line real-time meteorological data, ..) was very similar to RODOS.

Head of project 3: Dr. Müller

II. Objectives for the reporting period

The main aim for the reporting period was the further development of the modules for food chain transfer of radionuclides and for dose calculation in order to meet the requirements of the RODOS system in its prototype 2 version presently under development. In this field, the results of the modules to be presented to the user and the interfaces with other modules of the system had to be defined in detail to direct the program development to that goal. This requires close collaboration with those institutions (KfK, NRPB) who are developing the countermeasure modules for immediate and long-term actions.

In parallel to this the development of a methodology for providing information on the uncertainty of the results has been started by performing an uncertainty and sensitivity analysis for different radioecological scenarios.

III. Objectives for the next reporting period

The adaptation of the program modules according to the endpoints agreed has still to be performed rather intensively during the next working year. Several programs of the dose module have to be altered in a way that they can be used by different modules. In some parts this requires the development of an entirely new structure of the programs.

Two exposure pathways will be introduced in the dose module which have not been considered so far: inhalation by resuspended radioactive material, and external exposure from radionuclides deposited on the skin and on clothes.

The development of a methodology for providing uncertainty information will be continued but with less priority than the above tasks. In this area, an algorithm which allows to give some quick indication about the uncertainty of actual model results to the user of the RODOS system will be worked out.

IV. Progress achieved including publications

During the previous funding period, the module package ECOAMOR (ECOSYS ASY Modules for RODOS) which comprises a foodchain transport module and a dose module for the analysing subsystem (ASY) of RODOS was designed, developed and integrated into the prototype version 1 of RODOS. In this version, the

calculations of specific activities in foodstuffs and ingestion doses were restricted to those pairs of nuclides and foodstuffs with the highest contribution to the ingestion dose in order to reduce calculation time and computer memory space. For the same reason the results were calculated only for a subgrid of up to 11 x 11 grid points (out of the atmospheric dispersion data grid with 41 x 41 grid points). Due to this reduction of grid points the graphical output of the ECOAMOR modules was felt to be unsatisfactory in its spatial resolution.

The program development in the reporting period was aimed to remove these shortcomings. Simply increasing the spatial dimensions of the modules leads to an enormous increase in the demand for storage space and computing time which is expected to exceed the limitations of a real time system with a cycle time of 10 minutes.

For this reason, another calculational approach for foodchain transport and ingestion dose had to be developed for ECOAMOR. In this approach, the calculation of specific activities in feeds and foodstuffs, the activity intake and the resulting ingestion dose is first performed for a normalized deposition onto the different types of deposition areas (i.e. the surface of different types of plants, and the soil). Then these normalized values are used to evaluate the specific activities and ingestion doses for a great number of locations.

This approach leads to a reduction in computing time by a factor of about 5 and a similar reduction factor in storage space for intermediate results. This allows the calculations to be performed with the desired high spatial resolution. Other advantages of the normalized calculation are an improved flexibility in the creation of results, the inclusion of all nuclides and types of foodstuffs and the possibility of getting results for groups of nuclides (e.g. iodine isotopes) and/or foodstuffs (e.g milk and milk products) with little expenditure. On the other hand, the disadvantages are that the specific activity in foodstuff is not the real interface between food- stuff transport module and dose module, and products which are based on more than one deposition area (i.e.which are produced from different types of plants) cannot be considered without further extension of the calculation procedure. But the latter problem seems not to be a severe one.

Further development of the program modules will be necessary after final agreement on the results to be presented to the user and on the interfaces of the ECOAMOR modules with the countermeasure modules developed by other contractors. In this field, a new concept for the presentation of the results has been worked out which gives high flexibility to the user in defining the output of the

RODOS analysing subsystem in the automatic and the interactive mode. For this purpose a detailed compilation of the results and interfaces has been set up which has to be further discussed with the developers of the RODOS operating system and the countermeasure modules.

The data base of dose conversion factors has been extended to include all organs. The data sets used in the food chain calculation have been partly updated due to the recent development of the radioecological model ECOSYS-87, which is the basis of the ECOAMOR modules.

The development of a methodology for providing information on the uncertainty of the results of the food chain and dose modules has been started in the reporting period. For this purpose the radioecological model ECOSYS-87 has been linked with the program package PRISM. The latter is a code developed by Oak Ridge National Laboratory for estimating the parameter sensitivity and uncertainty of model results based on Monte-Carlo-method using Latin-Hypercube sampling. This required to estimate the range of uncertainty for each of the relevant model parameters.

Uncertainty estimations have been made for different scenarios, considering different radionuclides, types of deposition (dry, wet), times of deposition (winter, early and late summer) etc. For each scenario the ranges of uncertainty (given by frequency distributions) of the calculated foodstuff contaminations and the ingestion dose have been determined, and those model parameters contributing most to the uncertainty have been identified.

These investigations will be continued in the future considering a much wider spectrum of model scenarios. After that, the resulting data base with uncertainties of model results will be used to develop an algorithm which allows to give some quick indication about the uncertainty of actual model results to the user of the RODOS system.

Publications:

[1] Mueller H., Friedland W., Proehl G.:
Uncertainty in the ingestion dose calculation.
Accepted for Publication in Radiation Protection Dosimetry

[2] Friedland W., Mueller H., Proehl G., Brown J., McColl N.P., Jones J.A., Haywood S.M.:
Modules for foodchain transport, dose assessment and long term countermeasures in RODODS, the European decision support system.
Accepted for Publication in Radiation Protection Dosimetry

Head of Project 4: Professor French

II. Objectives for the period

The primary aims of the contribution are:

1. The development of Bayesian modelling techniques to forecast and handle uncertainties in RODOS, initially focusing on the inclusion of uncertainty measures within the plume forecasting module (using the Rimpuff model) of the ASY subsystem. Specifically the following will be investigated and implemented.
   - assimilation of monitoring data;
   - estimation of release height;
   - reporting of means and variances of predictions at given sites;
   - estimate the source term with allowance for autoregression;
   - make allowances for modelling error;
   - consideration of the computational advantages of working on a variable grid.
2. Subsequently, attention will widen to consider uncertainty measures in the forecasting of the efficacy of countermeasures. We will consider passing appropriate measures of uncertainty between modules in RODOS and providing an audit trail to justify the predictions made by the system.
3. The development of methods to communicate uncertainty to non-numerate decision makers.

In addition to the above, Leeds and Warwick will also contribute general expertise on decision analysis, statistics and uncertainty modelling, numerical methods and software engineering.

III. Progress achieved including publications

Against the objectives we have achieved the following.

1. Bayesian updating techniques have been incorporated into the latest version of Rimpuff and the code optimised somewhat. The code is also being modified so that it uses monitoring data to:
   - estimate the source term with allowance for autoregression;
   - allow for unknown source height by mixing three Rimpuff models;
   - allow for uncertain wind direction by mixing three Rimpuff models;
   - pentify puffs with respect to (local) wind direction not grid co-ordinates.

   The modifications are made and are being tested against old and new data sets (e.g. Barsebeck and Guardo).

   Some theoretical work has pointed to the possibility of considerable computational advantages and enhanced statistical performance being obtained by reorganising the way that puffs are indexed and stored in Rimpuff. This will enable all the techniques currently bring developed in influence diagram and belief net theories to be deployed. A start has been made in recoding the main routine in C++ (i.e. using object oriented modelling).
2. Little has been done to address this objective yet: work is planned for Autumn 1993 and Spring 1994.

3. The puff model and the associated uncertainty has been visualised using 3-d graphics. Animations of plume spread can now be generated on Silicon Graphics machines. It is unlikely that this work will be incorporated in the versions of software to be delivered by Spring 1994 for HP machines; but it will act as a demonstrator for future development. Work is planned for Autumn 1994 to develop simple statistical plots to demonstrate the uncertainty to decision makers under the X-Windows graphical interface. This should be available within the software to be delivered in Spring 1994.

Publications

[1] French and Smith (1993) "Using monitoring data to update atmospheric dispersion models with an application to the RIMPUFF model." *Radiation Protection Dosimetry* (In press)

[2] Smith and French (1993) "Bayesian updating of atmospheric dispersion models for use after an accidental release of radiation" *The Statistican* (accepted for publication)

[3] French, Ranyard and Smith (1993) "Models, data and expert judgement in decision support systems" Institute of Nuclear Engineers Conference, Glasgow, September 1993. (Accepted for publication in proceedings)

Future objectives

Continue with further work on objectives 1, 2, 3 plus

4. Fully introduce object oriented code into Rimpuff allowing full use of the influence diagram and belief net algorithms which will improve performance and allow enhanced statistical analysis and uncertainty handling.

5. Develop software to handle uncertainty within the CSY subsystem (i.e. begin implementing some of the theoretical work under objective 2).

Head of project 5: Dr. Sohier

II. Objectives for the reporting period

The development of a methodology based on a comparison between early monitoring data and model predictions to reduce the global uncertainty about the radiological situation during the initial phase of a nuclear accident.

An algorithm was was developed to quickly reduce the uncertainty of the source term based on the utilization of fuzzy logic in case of simple situations (simple topography - simple meteorological conditions - simple source term) and was tested versus tracer experiments (passive pollutant).

In parallel to this, collaboration with other institutes to define specific training courses for the application of RODOS as a training tool and to define the mode of utilization of RODOS in such courses.

III. Objectives for the next reporting period

Consolidation of methodology and algorithm which was hitherto developed. Application to simple topography and meteorological situation but consideration of more realistic source terms : nuclide mix and time dependency. Further development of data assimilation module to consider every kind of early data. Application of methodology to available contamination data bases (e.g gamma dose rates available at NPPRI-Trnava).

Translation of PC-DOS software to UNIX-environment considering the RODOS boundary conditions.

Independently of this, collaboration with other involved institutes for the development of illustrative scenarios and course material for inclusion of RODOS in specific training courses.

IV. Progress achieved including publications

A clear and comprehensive picture of the actual and future contamination in the environment in case of a nuclear accident is a prerequisite before any sound decision about protective actions to the population can be taken. This work is concerned with the analysing subsystem ASY of RODOS to obtain a continuously increasing confidence in the picture of the radiological contamination. Only the

initial phase (during and shortly after emission) when the rapid introduction of appropriate countermeasures in the near range are of uttermost importance is considered. Several kinds of information will lead to reduction in the initial uncertainty , such as :

- pre-calculated scenarios
- in-plant and on-site data
- meteorological data & environmental data
- pre-calculated scenarios

This work is mainly concerned with the continuous comparison of model predictions, using meteorological data, with available monitoring data in the near range.

Our work performed in the framework of the contract BI6-106-B between the SCK/CEN and the CEC has indicated some basic difficulties in performing such an optimization. For example, unless a careful monitoring programme is executed, it is difficult to guarantee a unique solution. A method based purely on the adaptation of model input parameters with the help of Monte-Carlo calculations will be very time intensive and will possibly generate non physical solutions.

To tackle these difficulties some assumptions were made, assumptions to be relaxated in a later phase. Only a simple dispersion model (Gaussian-like trajectory model) is used to cope with simple situations (simple topography, meteorological situation and source term).

A routine on IBM-PC has been built to quickly obtain a best estimate of the source term as well as the wind direction and effective plume height using near-range (up to several km) data.

The basic quantity is the individual (P/O)-ratio (Prediction/Observation) for each monitoring point. Depending on its value, a (P/O)-ratio can be defined to belong to a discrete class represented by a class number and related to the quality of the ratio. If done classically, some information is lost because of the sharp transition in class number for the (P/O)-ratios near to the boundary of a class. For this reason a fuzzy set approach was introduced. For each class (5 in total : Very Good, Good, Medium, Poor and Very Poor) a membership function was defined giving rise for each (P/O)-ratio to a sequence of 5 values which represent the degree of membership to each class (ref. 1,2). For each (P/O)-ratio the quality of this ratio is obtained by the class number (0 : VG ... 4 : VP) by selecting the class with the highest value for the membership function.

The routine is divided in two parts (I and II). Part I tries to obtain as quick as possible an approximate value for the main model parameters (source term, wind direction and effective height) while part II allows for a possible refinement. Part I-1 realizes a regression in order to deduce an approximate value for the effective height based on the behaviour of the concentration downwind (e.g. localization of the maximum concentration). Part I-2 makes an optimization on the source term, the wind direction and the effective height. The source term is calibrated by minimizing the average of the class number of each (P/O)-ratio. An adaptable multiplication or reduction factor is used to accelerate the process. The wind direction is optimized by comparing the partial averages of the class number for receptors on both sides of the supposed wind direction. The wind direction is allowed to vary with distance. The optimization cycle is stopped if the difference of the values of two successive VG membership functions averaged over all monitoring points differ less than a predefined value, on the condition that the average VG membership function tends towards one.

In part II successive smaller intervals are defined around the best estimates of the source term and wind direction obtained in I. Calculations are performed for sets of values within this intervals using the "global quality" (VG membership function, averaged over all monitoring points) as an optimization criterion.

Up to now the routine has shown to be operational, when tested with available tracer experiments (Mol, Karlsruhe), for a passive contaminant for a single release. When the effective height is supposed to be known a standard error (100 * abs(best-estimate source term - real source term)/ (real source term)) lower than 200 % can always be obtained in part I and can usually be reduced to 50 % in part II. Supposing an unknown release height the standard error can still be reduced to 200 % in most cases but part II does not give rise to a significant improvement. One of the difficulties which has not been resolved up to now concerns the definition of appropriate weighting factors for points lying at a certain distance from the plume axis to take the limited validity of the model far away from the plume axis into account.

Independently from the previous part, collaboration with other institutes has been initialized to define specific training courses for the application of RODOS as a training tool and to define the mode of utilization of RODOS in such courses.

Publications

[1] Sohier A.,Govaerts P.
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[2] Sohier A.,Van Camp M.,Ruan D.,Govaerts P.
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[3] Van Camp M.,Ruan D.,Sohier A.,Govaerts P.
The use of fuzzy set theory to reduce uncertainties on the source term and the wind direction in decision aiding systems.
Joint International Conference on Mathematical Methods and Supercomputing in Nuclear Applications, April 1993 Proceedings vol I, 432-442

[4] RODOS(B)-TN(93)01
RODOS Subtask "Training": Potential implementation of RODOS in the Mol Training Courses, 26.11.92 SCK/CEN

Head of project 6: Ms. Haywood

II. Objectives for the reporting period

The first objective for the current reporting period has been to extend the development of the food countermeasures model, following the implementation of a prototype version of this model into RODOS-PRTY1.0 in 1992, and to develop countermeasures models for relocation and decontamination. The second objective has been to develop a structure for the long-term countermeasures module (FRODO) of RODOS in which to incorporate these countermeasures models and to agree with other module developers the interfaces between the FRODO module and adjacent modules of RODOS, and the way in which FRODO is utilised within the RODOS system.

III. Objectives for the next reporting period

The objective for the next reporting period is to continue the development of the FRODO countermeasures module for food countermeasures, relocation and decontamination. The countermeasures module will then be implemented into the 1994 version of the RODOS system in collaboration with KfK, GSF and other organisations. Work will continue on the development of the required countermeasures databases for use with the FRODO module which will incorporate the data from the collaborative research with institutes in the CIS on agricultural countermeasures.

IV. Progress achieved including publications

The work at NRPB during the reporting period has concentrated on the development of countermeasures models for assessing the consequences of long-term countermeasures options. The countermeasures options under consideration are food countermeasures, relocation and decontamination. The other major aspect of the work has been the development of a module structure for implementing these countermeasure models into RODOS. In particular emphasis has been placed on developing a flexible framework that can be further developed in the future under the RODOS framework.

Model Development

NRPB produced an example module for the consideration of food countermeasures related to milk and milk products as part of the prototype version 1 of RODOS presented at Schloss Elmau in October 1992. Emphasis has been placed in this reporting period on extending the models developed for this prototype module to be applicable for the major food groups, eg. green vegetables, root vegetables, cereals, milk and meat, and to take into account a wide range of countermeasure options both for the short term following an accidental release to atmosphere and also in the longer term.

The FRODO module will be directed to answering different types of question in the first few days after an accident than those that might be considered in the longer term when more time is available to decide on the best options for the situation being evaluated In the first few days the module will be directed to answering scoping questions advising the RODOS user on the scale of the problem. If food is required to be banned based on a set criterion the module will consider what actions can be taken to avoid banning food and, if a ban cannot be avoided, will then consider what can be done to reduce the length of ban required and the amount of produce affected. The module will include models for agricultural countermeasures including food disposal, food processing, food storage and the feeding of uncontaminated feed to animals, to provide a robust model that can be applied to all foods considered. The user will therefore receive an idea of the scale of the problem and the options available to alleviate the problems in the short term. In the longer term the FRODO module will consider a much wider range of food countermeasure options. It is the intention that these options will be assessed, in the later decision aiding modules of the RODOS system, in terms of their effectiveness and feasibility and that this assessment will be made partially on the basis of information provided to these later modules by FRODO. Countermeasure options such as amelioration of soils, change in crops grown and change in land use will be addressed in FRODO. The module will be directed towards answering questions concerning the longer term options to reduce doses and activity concentration levels.

Models for determining the requirements for, and the consequences of, relocation have been developed in this reporting period. Two types of relocation have been considered, namely temporary relocation and permanent relocation. Permanent relocation is the removal of people from an area with no expectation of their return, although the land may be released at a much later time and subse-

quently resettled by different individuals. Temporary relocation is the removal of people for an extended but limited period of time. The consequences considered in FRODO relate to the areas of land interdicted, the time periods over which this occurs, the numbers of people relocated, and the doses saved as a result of relocation. Models for assessing the direct effect of decontamination on doses and also the impact of decontamination on the requirement for relocation and agricultural countermeasures are also being developed.

During the reporting period the development of databases containing information on the effectiveness of countermeasures and the associated costs and equipment required etc. has been ongoing. The data used in the food countermeasures models will, in general, be in the form of reduction factors that can be applied directly to the concentrations in the foodstuff or empirical models that can be used to modify the animal diets, levels in soil/crops or concentrations in animal products following the giving of additives in the feed. These databases are being developed in close collaboration with the CEC/CIS Joint Study Project 1 participants in the Ukraine where data on the effectiveness of food countermeasures and other relevant data are being collated.

Module Structure

FRODO will be sub divided into a series of sub-modules considering the three countermeasure options; food, relocation and decontamination. The relationship between these sub-modules is illustrated diagrammatically in Figure 1. The broad functions of each sub-module are described below.

FRODO-CONT	- controls and directs the other sub-modules
FRODO-RELOC	- assesses the consequences of relocation
FRODO-FOOD	- assesses the consequences of food countermeasures
FRODO-DECON	- assesses the consequences of decontamination
FRODO-SUMM	- manipulates output from the other sub-modules for transfer to other modules of RODOS

The structure of FRODO has been developed to enable the countermeasure options to be considered separately and provides a flexible structure into which other models or sub-modules could be added in the future.

Interactions with other module developers

NRPB attended a coordination meeting on dose and countermeasure models and links with decision expert systems in February 1993 at KfK. Discussions were held

on the planned developments of the dose and countermeasures modules which are closely linked and in particular, the requirements of the two modules were identified and the interfaces between them discussed. Close interaction between the developers of the dose module and NRPB has been maintained over the reporting period to ensure a consistent approach where appropriate and to resolve problems concerning the integration of the modules together and into the RODOS system within the next reporting period. Contact has also been made with the developers of the other RODOS modules from which FRODO requires data and those which require endpoints calculated by FRODO, to enable the process of defining interfaces to commence.

Publications

[1] Friedland, W., Müller, H., Prohl, G., Brown, J., McColl, N.P., Jones, J.A. and Haywood, S.M.,
Modules for foodchain transport, dose assessment and long term countermeasures in RODOS, the European decision support system.
Rad. Prot. Dos. Special edition on Third International Workshop on Real-time Computing of the Environmental Consequences of an Accidental Release to Atmosphere for a Nuclear Installation, Schloss Elmau, October 1992 (to be published).

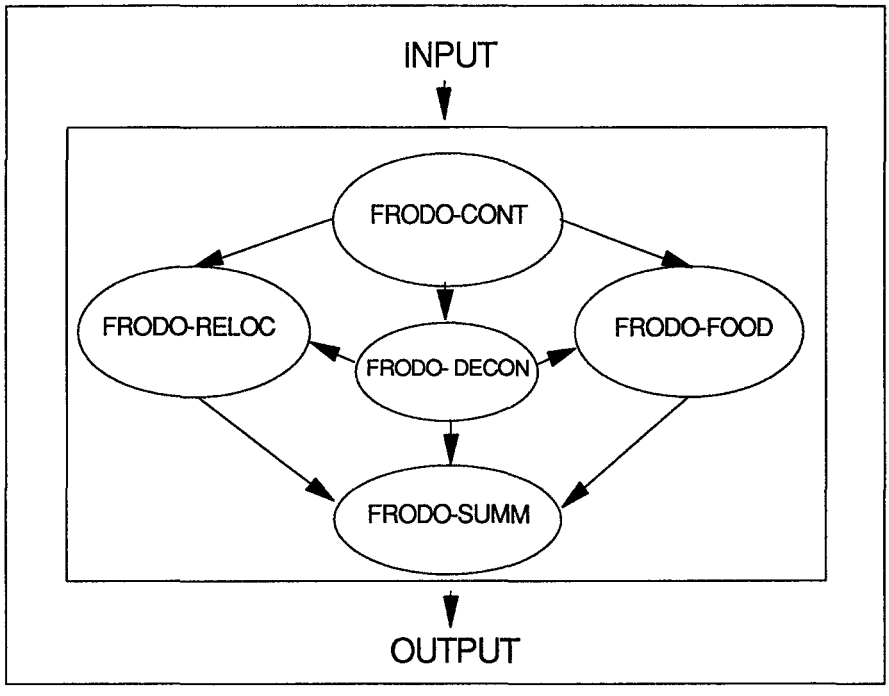

Figure 1. Proposed Internal Structure of the FRODO module.

Head of project 7: B. Bleasdale

II. Objectives for the reporting period

The objectives for the current and the next reporting period are identical and are:

1. Advise and support the project in matters relating to training and exercises.
2. Ensure the practical lessons learnt from Nuclear Electric's extensive experience in scenario development and exercise programmes are fully utilised.
3. Ensure that a systematic approach to training is used for the project.

III. Progress achieved

1. Participation at the subgroup meeting on "training" held in Karlsruhe in September 1992 and Paris in April 1993 including preparation of material in advance for discussion at both meetings.

2. Production of description of training courses in the UK where RODOS may be utilised, identifying for each course:

   Frame, aim and target group
   Duration and division
   Participants and their background
   Lecture content
   Nature of any exercise included in the course

   Additionally providing for each course an evaluation with respect to the use of RODOS within the course.

3. Utilising the systematic approach to training, production of a draft modular training package structure, identifying for each module learning objectives and assessment criteria, and developing the associated matrix of modules required for each of the target groups for the training.

4. Development and provision to the group of typical lesson plans.

5. Work currently in hand, and at an advanced stage includes

   - preparation of potential accident scenarios and source terms;

- description of benefits and drawbacks of countermeasures with respect to their effectiveness against different exposure pathways and their impact on decision making;
- monitoring strategies.

Progress Report

Contract: FI3P-CT920038 **Sector:** C24

Title: Deposition of artificial radionuclides, their subsequent relocation in the environment and implications for radiation exposure.

1)	Jacob	GSF
2)	Roed	Lab. Risø
3)	Brown	NRPB
4)	Goddard	IMPCOL
5)	Roed	Lab. Risø

I. Summary of Project Global Objectives and Achievements

A Global Objectives

To improve where necessary the models and parameterizations used in estimating the intensity and spatial distribution of deposited activity and the total health/economic impact of such deposits in assessments of the consequences of accidental releases of radioactivity, and to this end to attain a better understanding of:

- the influence of various weather conditions on deposition, particularly weather conditions which can lead to high deposition fluxes such as fog, snow or intense rain;
- the skin deposition velocity, for a range of aerosol sizes, in controlled environments and under normal conditions;
- the indoor air concentration and deposition for micron and sub-micron-particles. The latter will be achieved by developing new methods for labelling, dispersing and measuring sub-micron particles, labelled with neutron activatable tracers;
- the weathering of deposits in urban and rural environments and its impact on long-term external exposure;
- resuspension of deposited ^{137}Cs activity;
- the ultimate fate and dosimetric impact of radionuclides carried by run-off water.

A new model will be developed calculating the inhalation dose, skin dose and the external dose originating from indoor deposits and based on data concerning the cloud of pollution and the dwelling in question.

An additional objective is to review and consider the results from the experimental programmes with a view to the future incorporation into accident consequence assessment codes and computer support systems for use in emergency planning and response.

B. Summary of achievements

a) Wet deposition of atmospheric aerosol

A fog water collector (Rotating Arm Collector - RAC) was developed to sample airborne fog water in different size fractions. During foggy nights aerosols were sampled size fractionated and the deposition on a polyethylene surface and on grass was measured. The deposition velocity on grass was determined for six elements. Ca, which was found preferably on large aerosols (MMD in the range of 1 - 3 μm), had in the average a deposition velocity of $2\ 10^{-2}$ m/s, the other five elements with MMDs of 0.2 -1 μm had average deposition velocities of 1.10^{-3} to 4.10^{-3} m/s. Compared to dry depositions these preliminary results are larger by one-and-a-half order of magnitude for Ca and by half an order of magnitude for the other elements.

b) Deposition on skin

For test chamber research directed towards skin deposition, a new technique has been developed based on the principle that the difference between aerosol mass flux as indicated by air concentration time decay and by wall surface sampling must be the flux to any object introduced into the chamber. An initial test using an inflated balloon (which was itself sampled by surface wipes and by bulk activation analysis) gave excellent results.

During an experimental campaign in a Danish house some preliminary measurements of aerosol mass deposited on skin and hair were made in order to establish detection limits. The results indicate that without exceeding the aerosol concentration at which coagulation might occur, a detectable mass of tracer can be measured on hair and skin.

c) Labelling and dispersion of sub-micron monodisperse aerosols

Attempts to label polystyrene latex particles with indium (a sensitive activation tracer) were not feasible due to an organic indium complex in liquid form not being available. Generation of indium particles using a condensation generator was not successful due to decomposition of the material at the high temperatures of the available furnace (a commercial low-temperature furnace is a future option). A successful method was the nebulisation of a suspension of indium acetylacetonate (from acetone/ethanol) with a 3-stage cascade centripeter placed at the generator outlet to remove large particles. These particles have been used both in the test chamber and in a house experiment.

d) Indoor air concentration and deposition

Deposition velocities to individual indoor surfaces (wallpaper, aluminium, carpet) have been investigated in the test chamber, whereby samples are attached to test chamber surfaces and deposition velocities derived from the change in time decay parameter. An expressed dependence of the vertical deposition velocity on the surface roughness was observed for all of the three particle sizes used in the experiment (2, 4, and 6.5 μm). The roughess of the surfaces is characterised by friction velocity measurement in a wind tunnel.

In spring 1993 a new house became available in the village of Ferslev in Denmark. During a two week experimental campaign the new sub micron particle equipment could be used successfully. In general, deposition velocities increased with particle size and degree of furnishing, just as expected. The obtained results were in good agreement with earlier results. The experiments confirmed that indoor deposition rather than filtration over the building

envelope is the factor of importance when determining concentration of indoor air particulates of outdoor origin. Comparisons of two methods to determine indoor deposition (deposition on surface samples and decrease of the tracer concentration in air) indicated error sources in some tests and improved the credibllity of results in the other test.

e) Weathering of ^{137}Cs in urban and rural environments

The effect of surface roughness and migration of caesium into soil on the gamma-dose rate in air over grassland has been studied by two methods. The evaluation of *in situ* gamma-ray spectrometric results and measured activity distributions in the soil showed only negligible differences for the observation period of six years after deposition. Systematic differences were found between the migration behaviour of caesium in Southern Bavaria and Ukraine. For the observation period 3 to 6 y after the deposition, the gamma-dose rate in air per unit deposit was about 40% higher in Ukraine. A correlation analysis indicated the sand content, the cation exchange capacity, the pH-value, the annual precipitation, the physical-chemical binding of the radionuclides in the deposit and the type of deposition (dry or wet) as possibly important parameters for the migration process.

A field campaign in the heavily contaminated area of Gävle in north-east Sweden was carried out. Very little, if any, decrease in the levels of radiocaesium on walls of buildings in the Gävle area identifiable 7 years after the accident. The levels of radiocaesium on asphalt surfaces have now decreased to less than 10% of what was measured in 1988. The levels were now below the detection limit. As for the concrete paved surfaces, the remaining 10% of the initially deposited radiocaesium seems to be firmly fixes. No significant decrease has been found over the last 2 years. On the grassed surfaces, the unscattered photon flux decreases as could be expected (more or less according to the formula derived by Gale et al. in 1963).

Measurements of the weathering effects on clay roof tiles from Gävle confirmed an analytical approximation (half of the deposited material decreasing with a weathering half-life of $1.5\ 10^3$ days and the other half with $3.4\ 10^2$ days), as it was assumed in the computer code URGENT which has been developed at Risø to give estimates of accident consequences. The caesium level on the tiles contaminated in Roskilde decreased a little faster.

f) Resuspension of deposited ^{137}Cs activity

The data from Goiânia show a significant seasonality and a slow long-term decrease with time for the activity concentration in air and deposition rate. Resuspension factors in the order of 10^{-8} to 10^{-9} m^{-1} (based on the air activity data) were derived. A wide range of values for nominal deposition velocities were found, averaging at 5 to 6 cm s^{-1}. Impactor measurements indicate that about 30 to 60% of the total particulate matter in air is due to aerosol particles above 15 μm diameter. The data as a whole suggest that in Goiânia resuspension and deposition are mainly local phenomena and no evidence was found for a significant spreading of ^{137}Cs from the place of primary contamination.

A new series of experiments was recently initiated in which stratospheric Be-7 is used as a tracer to determine the size distribution of resuspended particles from a field. The sampling was carried our using two Berner impactors at different elevations.

g) Consideration of experimental results for external exposure and other irradiation pathways in the context of accident consequence assessments and real-time response systems

The work during the reporting period has concentrated on the review of the current status of experimental work in the areas of deposition of artificial radionuclides and their subsequent relocation in the environment. Advice on the modelling approach and parameter values for external gamma dose and inhalation dose calculation for use in accident consequence assessment codes has been given based on the current status of the experimental work. Contact has been made with participants in the CEC CHECIR project in connection with long term countermeasures and, in particular, decontamination in rural and urban environments.

Head of project 1: Dr. Jacob

II. Objectives for the reporting period

- Development of a fog water collector, sampling fog water in different size fractions
- Measurement of the fog water deposited on a grass surface
- Measurement of element specific size distributions of aerosols
- Determination of element specific deposition velocities during fog events
- Analysis of measurements on the attennation of the gamma dose rate in air due to the migration of caesium into soil
- Analysis of resuspension and redeposition measurements of caesium in Goiña

III. Progress achieved including publications

The deposition process of aerosol by fog water has been studied by field measurements with different devices. Shortly before the fog events, aerosol samples were collected size fractionated on polypropylene foils of 8 µm thickness using an eight stage Berner impactor. Sampling times were between 2 and 4 hours. The Liquid Water Content (Mass of water per volume of air [g/m^3]) of the fog air is assumed to be an important parameter for the deposition process. It was measured by an optical device (PVM, Gerber Scientific).

A fog water collector (Rotating Arm Collector - RAC) was developed. With this device it is possible to collect fog water in different size fractions. The collection of fog water samples with different cut off_{50} (radius of droplet that is collected with an efficiency of 50%) can be achieved by changing the rotation frequence of the separator. With an arm of the length of 1m on every side and a separator (PVC rod) of a diameter of 6 mm, the cut off_{50} can be modified from 4 to 10 µm (**Figure 1**). An additional modification of the cut off_{50} can be achieved by the use of a shorter rotating arm of 30, 50 or 70 cm or (and) a change of the separator geometry. The fog water is collected from an air volume of up to 3076 m^3/h which means that in a fog with a Liquid Water Content of 0.1 [g/m^3] the sampling time for 10 ml of fog water will be 4 minutes, for a sampling efficiency of 50%.

Deposited rime ice or fog water has been collected on a polyethylene surface of 0.25 m^2 diameter which is opened in the evening shortly before the begin of the fog event and closed in the early morning. The water and ice samples are scraped into a sampling bottle from polyethylene fixed at the lower side of the surface using a polyethylene scraper. Fog water deposited on a natural surface (a grass surface of 0.42 m^2 was used) is registered by a balance lysimeter consisting mainly of a very sensitive balance (Mettler) which allows a weight resolution of 1 g. The grass surface grows in a pot with soil standing on the balance. The change of weight during fog events corresponds to the deposition of fog water.

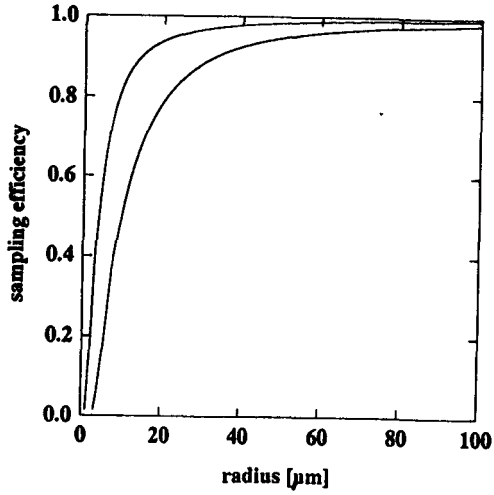

Figure 1: Sampling efficiency of the RAC at different rotation frequencies in relation to the droplet radius for arm radius 1m - Upper curve 400 rpm (cut off $_{50}$ 4µm), lower curve 50 rpm (cut off $_{50}$ 10 µm)

Aerosol samples are analysed for their elemental content using PIXE . Water samples are analysed for their elemental composition by ICP - AES (Inversed Coupled Plasma - Atomic Emission Spectrometry) and AAS (Atomic Absorption Spectrometry) after acidification of the samples (HNO_3 suprapur). Anions SO_4^{2-}, NO_3^- and Cl^- are analysed by IC (Ion Chromatography), NH_4^+ and pH value are analysed using electrodes. The trace element content of the aerosol is used as tracer for the deposition process.

Measurements during radiation fog events in autumn and winter show that the mass of water deposited to the grass surface depends on the Liquid Water Content of the air (**Figure 2**).

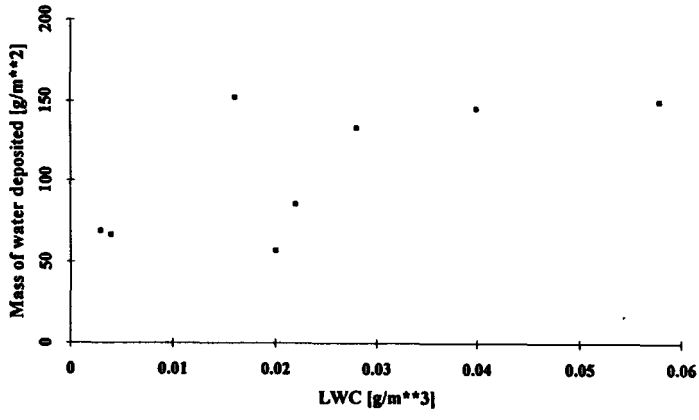

Figure 2: Mass of water deposited on the grass surface in relation to the LWC:
The two points with relatively high deposited water mass at low LWC might be due to a strong influence of condensation during fog events with a low density of fog.

Assuming that the aerosol particles are the only source of the elements in the fog water, element specific deposition velocities (v_d) can be calculated by

$$v_d = \frac{c_{aq} \cdot v_{aq}}{c_{aer}}$$

c_{aq} = concentration of element in fogwater [µg/ml],

v_{aq} = volume of water deposited per unit area and time [ml/m^2 s],

c_{aer} = concentration of element in airborne particles [µg/m^3].

Distribution parameter	Deposition velocity (m/s) Fe	Zn	Pb	S	Ca	K
mean	0.0027	0.0022	0.001	0.0017	0 .0184	0.0043
maximum	0.0063	0.012	0.0052	0.0075	0.029	0.024
minimum	0.0005	(0.00003)	0.0002	0.0003	0.0044	0.0004

Table 1: Deposition velocities (v_d) for particle bound substances deposited with fogwater during eleven fog events.

Deposition velocities for the elements analysed so far during nights with fog range from 3. 10^{-2} to 3. 10^{-4} m/s (**Table 1**) The deposition velocities would even be higher if samples were collected exclusively during the time with fog. Large deposition velocities are found for Ca due to the large MMD (mass median diamter - **Figure 3**) of the aerosol carrying this element. The water volume deposited per hour and m^2 of the grass surface (short grass with length of less than 5 cm) during the measurements ranges from 0.5 to 15 ml.

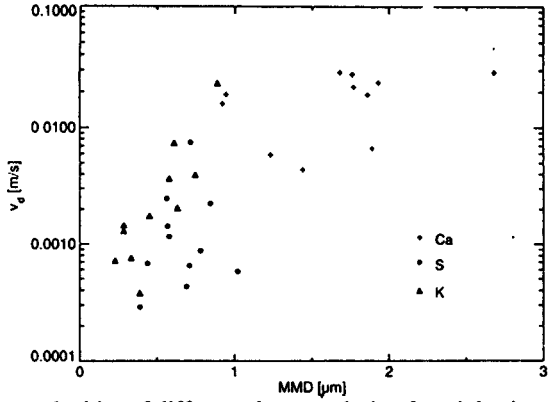

Figure 3: Deposition velocities of different elements during fog nights in relation to their mass median diameter on aerosols.

The effect of surface roughness and migration of caesium into soil on the gamma-dose rate in air over grassland has been studied by two methods. The evaluation of *in situ* gamma-ray spectrometric results and measured activity distributions in the soil showed only negligible differences for the observation period of six years after deposition. Systematic differences were found between the migration behaviour of caesium in Southern Bavaria and Ukraine. For the observation period 3 to 6 y after the deposition, the gamma-dose rate in air per unit deposit was about 40% higher in Ukraine (**Figure 4**).

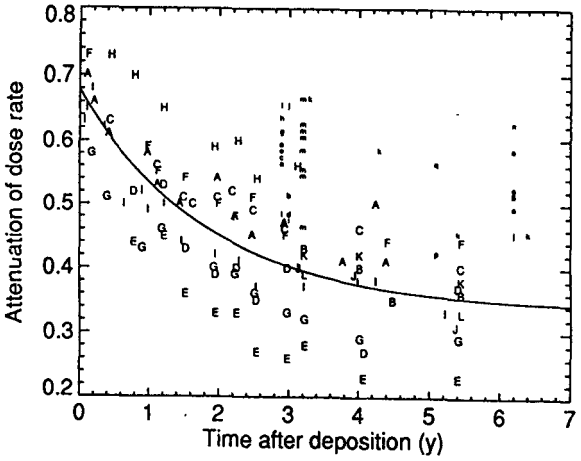

Figure 4: Attenuation of the dose rate in air relative to plane sources on a smooth air-ground interface. The capital letters indicate in situ gamma spectrometric results for Southern Bavaria. The small letters are results of depth profile measurements in the Ukraine and Russia. The line is a 3 parameter approximation of the data from Southern Bavaria.

The correlations between this attenuation and ten parameters characterising the deposition event, the soil and meteorological conditions have been analysed for two time periods after deposition (3 y and 6 y). Mathematically the attenuation factor was correlated ($f>0.6$) with the sand content and anticorrelated ($f<-0.6$) with the silt content, cation exchange capacity, pH-value, annual precipitation, distance from the release point, and the relative amount of wet deposition. For an evaluation of these findings it should be taken into account that several of the parameters are strongly correlated for the sites and measurements under consideration. The anticorrelation of the attenuation factor with the annual precipitation, the distance from the release point and the relative amount of wet deposition fits the general understanding of the migration. On the other hand, a correlation with the sand content is unexpected, but was also observed in another study (Tikhomirov, 1992).

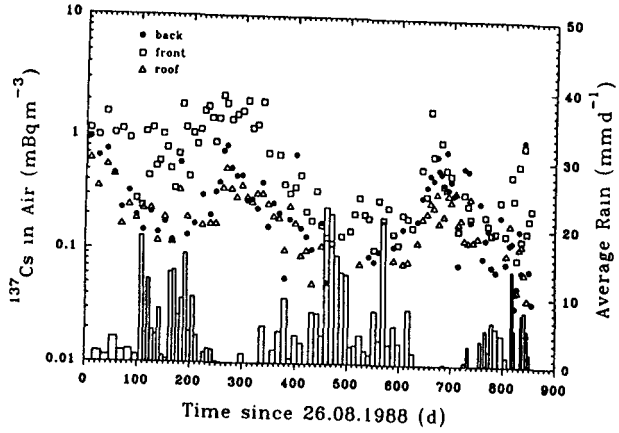

Fig. 5: ^{137}Cs air concentrations at three sites in Goiaña and measured precipitation rates.

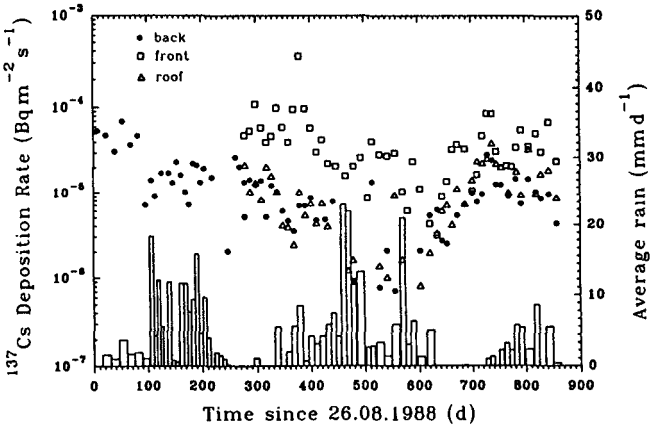

Fig. 6: ^{137}Cs deposition rates at three sites in Goiañia and measured precipitation rates.

From the evaluation of the environmental measurements performed in Goiañia, Brazil, after an accidental opening of a 137 Cs teletherapy source, following conclusions can be derived:

- The activity concentrations in air and deposition rates of resuspended ^{137}Cs in Goiânia, two years after the primary contamination, show a very slow long-term decrease with time but a significant seasonality (see **Fig. 5** and **Fig.6**).
- The results indicate that the resuspension and deposition of ^{137}Cs is a local phenomenon and resuspension factors of the order of 10^{-8} to 10^{-9} m^{-1} were derived for this scenario.
- No significant correlation between the air activity and its deposition rate was found and a wide range of elevated values for the deposition velocities was observed. The results confirm that in an urban area rather complex processes determine resuspension.
- Average nominal dry deposition velocities of about. 5-6 cm s^{-1} were found. The usual assumption of a deposition velocity of 0.1 cm s^{-1} for measured air activity concentrations would underestimate the actual deposition rates by a factor of approximately 50-60.
- Andersen impactor measurements indicate that about 30 to 60% of the total aerosol mass in air might result from aerosols above 15 µm diameter; these are not collected by the EPA-type air samplers.

- No significant spreading of ^{137}Cs throughout the city occurred during this study from the initially contaminated area by resuspension and air transport . This is to be expected from these measurements since most activity is associated with large particles with high deposition velocities.

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Jacob P., Meckbach R.: Recent developments in in-situ γ spectrometry. Proc. IRPA8, Worldwide Achievement in Public and Occupational Health Protection against Radiation, Montreal, pp. 305-308. International Radiation Protection Association, Montreal (1982):

Head of project 2: Dr. Roed

II. Objectives for the reporting period

1. Investigations of weathering effects on various urban surfaces in the town of Gävle in Sweden.
2. Investigations of weathering and wash-off of Chernobyl contaminated roof-materials from Roskilde and Gävle.
3. Investigations of downward migration of radiocaesium in soils.
4. Investigations of resuspension of deposited radioactive matter using rain-collectors of different elevations above ground.

III. Progress achieved including publications

INTRODUCTION

In this reporting period the progress achieved by Risø comprises a field measurement campaign to the heavily contaminated Gävle area in North-east Sweden. Also, investigations were carried out on contaminated roof materials from Chernobyl. Investigations have been made in the laboratory in order to investigate the fate of radiocaesium in soil. Further analysis of fresh samples from August 1993 will follow.

Weathering in the urban area

The wash-off and weathering on unpervious urban surfaces was investigated further by in situ gamma ray spectrometry in Gävle in late July 1993. This was the fifth measurement campaign conducted by Risø in this area, which is probably the most heavily contaminated outside the former USSR. The application of a lead shielded gamma spectrometer system which was used for these field measurements has been described in detail (Lit. 1).

As can be seen in Table 1, very little, if any, decrease in the levels of radiocaesium on walls of buildings in the Gävle area is identifiable 7 years after the accident. In one case, the level has actually increased since it was last measured. The change is not highly significant, but may be due to a wash-down of radioactive substances from the upper parts of the wall. The levels of radiocaesium on asphalt surfaces have now decreased so much that less than 10 % of what was measured in 1988 is left. This means that probably less than two percent of the initially deposited radiocaesium is still present on the road. The levels were now below the detection limit.

Table 1. ^{137}Cs levels measured in Gävle in 1987, 1988, 1990, 1991 and 1993 ($kBq \cdot m^{-2}$)

Surface type	1987	1988	1990	1991	1993
Plain red brick wall in 5-storey building (South-facing).	1.87 ± 9%	1.65 ± 9%	-	-	-
Plain red brick wall in 5-storey building (North-facing).	-	3.82 ± 7%	-	1.85 ± 7%	2.13 ± 15%
Yellow brick wall with roughened finish in single-storey building (South-facing).	-	1.03 ± 11%	-	0.79 ± 10%	0.76 ± 10%
Asphalt surface 5.8 m wide road.	-	1.48 ± 9%	0.44 ± 17%	-	-
Asphalt surface on crossroads.	-	1.19 ± 9%	0.50 ± 15%	-	-
Concrete paved area 1.	-	6.75 ± 6%	3.15 ± 7%	2.17 ± 8%	2.12 ± 9%
Concrete paved area 2.	-	9.71 ± 6%	4.26 ± 7%	3.13 ± 8%	3.10 ± 9%
Grassed area 1	-	113. ± 5%	117. ± 5%	-	66.4 ± 5%
Grassed area 2	-	85.2 ± 5%	66 9 ± 5%	58.7 ± 5%	51.3 ± 5%

As for the concrete paved surfaces, the remaining 10 % of the initially deposited radiocaesium seems to be firmly fixed. No significant decrease has been found over the latest 2 years. On the grassed surfaces, the unscattered photon flux decreases as could be expected (more or less according to the formula derived by Gale et al. in 1963). Soil cores (diameter 9 cm and thickness 10 cm) were collected from undisturbed grass areas in Gävle (Lit. 2) which have previously been examined in this way. The soil

cores were sectioned at intervals of 1 cm, dried at 50°C in an air oven. In the next few weeks the fresh samples will be analysed for their ^{137}Cs content in a high resolution (Ge) gamma ray spectrometer. The downward migration will be compared to results obtained from two previous campaigns in Gävle. The strength of fixation of caesium in the soil will be examined by sequential extractions, and also the fractions of caesium bound to different soil substances will be assessed.

Such investigations are important to evaluate the population doses from radiocaesium deposited in urban areas. The computer code URGENT, which has been developed at Risø to give estimates of the accident consequences based on experiments, revealed that in the urban centres, about 73 % of the total external dose can be ascribed to the open grassed surfaces, and in the suburban areas, where the shielding effect is smaller, as much as 89 % of the dose can come from contamination on grassed surfaces (Lit. 3). However, decontamination tests made during a field measurement campaign in the Chernobyl area in June 1993 showed that a simple digging of the ground could reduce the dose from the ground by some 74 %.

Table 2 shows the results of measurements to investigate the weathering effects on clay roof tiles. Sections A,B,C,D and E all represent clay tile roofs which have been contaminated by Chernobyl fallout in Gävle.

Table 2.

Weathering:

Tiles contaminated in Gävle:

section		july 90	feb 92	Corrected	Weathered-of
A	Cs^{137}	39,59	24,99	25.87	34,7%
	Cs^{134}	7,3	2,90	4,80	35,0%
B	Cs^{137}	38,52	26,15	27,07	29,0%
	Cs^{134}	7,17	3,10	5,13	28,5%
C	Cs^{137}	39,63	26,85	27,80	29,9%
	Cs^{134}	7,39	3,10	5,13	30,6%
D	Cs^{137}	39,61	27,08	28,04	29,2%
	Cs^{134}	7,38	3,22	5,33	27,8%
E	^{137}Cs				

Tiles contaminated in Roskilde

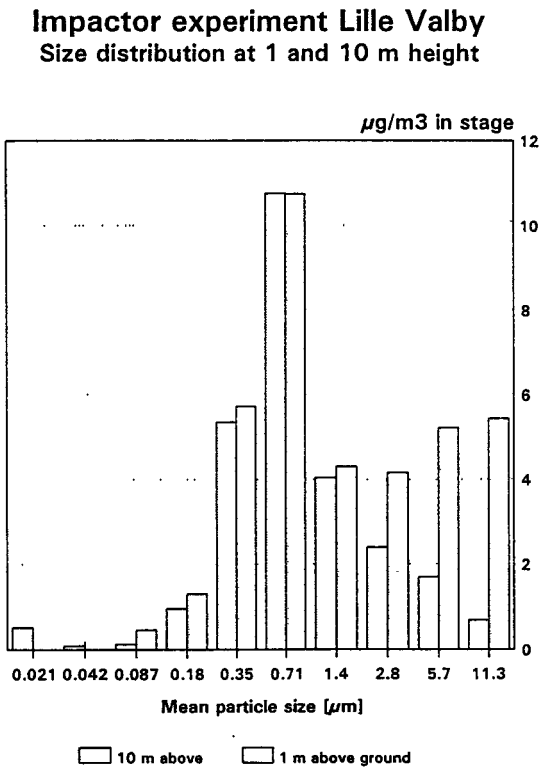
		27/4 88	10/2 92		
2.2 + 2.3 east	Cs^{137}	0,318	0,150	0,164	48,8%

The roofs have later been brought to Risø and reassembled to investigate the weathering effects on the roofs. The results from February 1992 have been 'corrected' for the decrease due to radioactive decay in the period since July 1990. As can be seen the results are fairly consistent. Further, it was found that the experimentally obtained data still corresponds fairly well to the data on which the URGENT model was established (half of the deposited material decreasing with a weathering half-life of $1.5\ 10^3$ days and the other half with $3.4\ 10^2$ days). The caesium level on the tiles contaminated in Roskilde decreased a little faster, but this may be due to the tiles being of a slightly different type.

The resuspension project, where rain-collection was carried out at two different elevations above ground and the samples measured for their content of radionuclides, has been continued. However, due to the many sources of uncertainty in such a project, further samplings will be needed to extract a resuspension factor from the obtained results.

A new series of experiments was recently initiated in which stratospheric Be-7 is used as a tracer to determine the size distribution of resuspended particles from a field. In the assessments shown below, in Fig. 1, the sampling was carried out using two Berner impactors at different elevations.

Fig.1.



List of publications

1) Roed, J. and Andersson, K.G., Using in situ gamma ray spectrometry to guide clean-up of Radioactive contaminated urban areas, presented at the 15th Mendeleev Congress in Minsk, May 24-29, 1993.

2) Andersson, K.G. and Roed, J., The behaviour of Chernobyl Cs-137, Cs-134 and Ru-106 in undisturbed soil: Implications for external radiation, Accepted for publication in Journal of Environmental Radioactivity, 1993.

3) Roed, J. and Andersson, K.G., Development of strategies for clean-up in urban nuclear contamination scenarios, to be presented at the International Symposium on Remediation and Restoration of Radioactive-contaminated sites in Europe, Antwerp, 11-15 Oct., 1993.

Head of project 3: Mrs. Brown

II. Objectives for the reporting period

The main objective for the current reporting period has been to review the current status of the areas of experimental work being carried out under this project. A second objective has been to look at ways of creating a forum in which the adequacy of current models can be evaluated and the results of the experimental programme can be reviewed, with the aim of reaching consensus between the contractors in the project. The nature of this work is such that much of it will be carried out in the latter part of the contract period.

III. Progress achieved including publications

The work at NRPB during the reporting period has concentrated on the review of the current status of experimental work in the area of deposition of artificial radionuclides and their subsequent relocation in the environment. Advice on the modelling approach and parameter values for external gamma dose and inhalation dose calculation for use in accident consequence assessment codes has been given based on the current status of the experimental work. Contact has been made with participants in the CEC CHECIR project in connection with long term countermeasures and, in particular, decontamination in rural and urban environments. The nature of the work of NRPB under this contract is such that the majority of the work will be undertaken under the next reporting period.

Experimental research in the areas of deposition of radionuclides and their subsequent relocation in the environment has been ongoing for several years. During the reporting period information from the contractors on the current status of this research, together with their experimental data, have been collected with the aim of determining ways in which these data could be used in the

models that are in current use. Many simplifying assumptions have had to be made in models in the past due to the lack of data on the behaviour of radionuclides in urban and rural areas. However, much of the experimental data available since the Chernobyl accident through research and measurement programmes has not been utilised in the models. The available data are now being collated, with the aim of forming the basis for evaluating the adequacy of the models currently used in accident consequence assessment codes and computer support systems for emergency planning and response, and considering ways in which the experimental data could be included in the models or used to improve the current models.

Advice was required for the accident consequence assessment code COSYMA, and the PC version of COSYMA, under development for the CEC, on the modelling of inhalation doses for people indoors. The current approach to modelling the reduction of air concentrations inside buildings based on experimental research was reviewed and, in collaboration with other contractors under this project, advice was provided on the best use of this experimental data in providing recommended parameter values for use within the current structure of COSYMA.

One of the objectives for this contract is to develop a forum to enable better communication between the modelling and experimental communities. NRPB attended a short progress meeting in July in Vienna where ideas for ways to achieve this were discussed. It was agreed that it was necessary to obtain consensus within the group of contractors on the modelling approach in the assessment and emergency response codes used. The group as a whole should review the adequacy of the current models for their given purpose and how the data from the current experimental research programme could be used to improve or support the current modelling approach. The reviewing of the current models will be carried out in the next phase of NRPB's work, to facilitate the start of a review of the adequacy of models and use of new experimental data by the group of contractors.

Publications

Brown, J and Jones, J A, Location factors for modification of external radiation doses, NRPB Radiological Protection Bulletin, No 144, July 1993.

Head of project 4: Prof. Goddard

II. Objectives for the reporting period

The general aims of the project are to address skin deposition of aerosols and to extend the range of aerosol measurements inside buildings to a wider range of conditions. This research will continue in close collaboration with Risoe, where the two organisations have demonstrated their ability to work together in an innovative way. During this first reporting period the following aspects have been addressed: continued application of Imperial College's expertise in labelling and measuring aerosols using stable tracer techniques together with the Imperial College's reactor; development of new techniques for labelling and generation of sub-micron aerosols; the use of the Energy Systems Section aerosol test chamber for a range of initial proving experiments relating to the measurement of aerosol deposition on skin.

III. Progress achieved including publications

Initial efforts centred upon the need to extend the sensitive methods of labelling surrogate monodisperse submicron aerosols. The importance of extending research to submicron aerosols lies in the corresponding deposition velocities being near the theoretical minimum, deposition being subject to phoretic effects and this size range being characteristic of long range transport. Attempts to label polystyrene latex particles with indium (a sensitive activation tracer) were not feasible due to an organic indium complex in liquid form not being available. Generation of indium particles using a condensation generator was not successful due to decomposition of the material at the high temperatures of the available furnace (a commercial low-temperature furnace is a future option). A successful method was the nebulisation of a suspension of indium acetylacetonate (from acetone/ethanol) with a 3-stage cascade centripeter placed at the generator outlet to remove large particles. These particles have been used both in the test chamber and in a joint house experiment at Risoe. Figure 1 shows the indium particle size distribution, as measured by an optical counter (complementary aerodynamic particle sizing was carried out at Risoe).

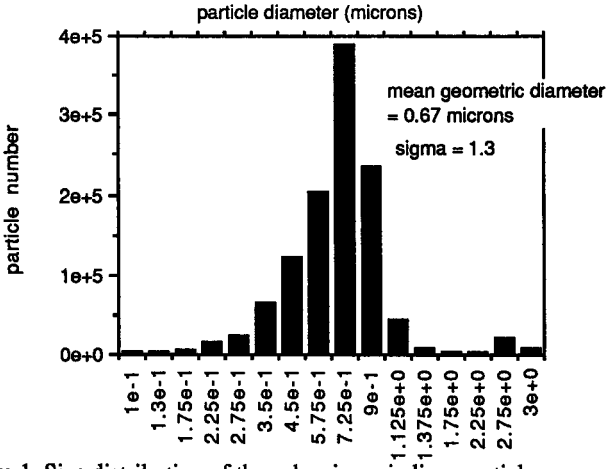


Figure 1. Size distribution of the sub-micron indium particles.

Test chamber research directed towards skin deposition studies has had two aspects: total deposition upon objects placed in the chamber and methods of sampling aerosols from indoor surfaces and clothing, hair and skin. Firstly, a new technique has been developed based on the principle that the difference between aerosol mass flux as indicated by air concentration time decay and by wall surface sampling must be the flux to any object introduced into the chamber. An initial test using an inflated balloon (which was itself sampled by surface wipes and by bulk activation analysis) gave excellent results, as shown in Table 1. In the next phase of this programme this method will be used to obtain bulk deposition to an anthropomorphic phantom to confirm the individual sampling of skin, hair and clothing.

Table 1. Example of the use of aerosol mass balance to predict the mass deposited on a balloon suspended in the test chamber.

	0.67 μm indium particles
Mass available for deposition on balloon and chamber surfaces (from decay curve)	55.7 +/- 0.2 μg
Mass deposited on chamber surfaces (from surface sampling)	53.5 +/- 5.1 μg
Estimated mass deposited on balloon (by subtraction)	2.2 +/- 5.3 μg
Measured mass deposited on balloon (by activation analysis)	2.2 +/- 0.01 μg

Deposition velocities to individual indoor surfaces, with a range of surface roughnesses, has also been investigated in the test chamber using a similar differential technique whereby samples are attached to test chamber surfaces and deposition velocities derived from the change in time decay parameter. Figure 2 shows the variation in vertical deposition velocity with surface roughness for three particle sizes. The roughess of the surfaces are characterised by friction velocity measurement in a wind tunnel.

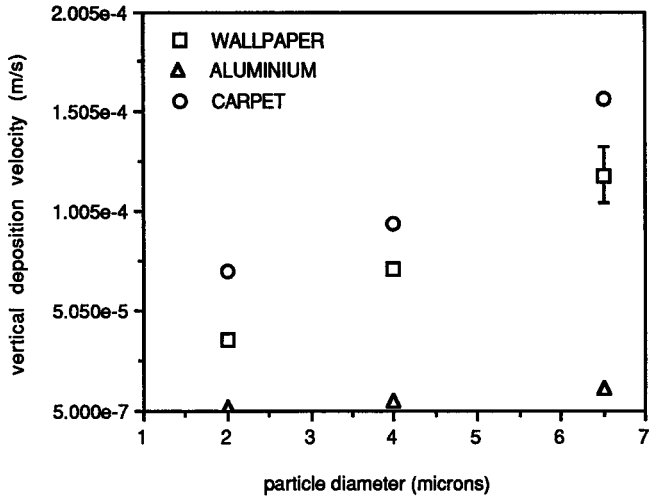


Figure 2. The variation in vertical aerosol deposition velocity with the surface roughness of one wall of the test chamber.

A range of investigations have been conducted preparatory to the selection of an anthropomorphic phantom, and into sampling methods for the surfaces of such a phantom and for human volunteer studies.

In collaboration with Risoe, a new joint experimental campaign was carried out in a Danish house. In addition to participating in the research and providing labelled silica aerosol, of sizes 2- 6.5 microns, Imperial College also provided the submicron aerosol referred to above. Both furnished and unfurnished room conditions were investigated and the existing data base of average indoor deposition velocities extended in terms of room geometry and aerosol size range. In order to establish detection limits, some preliminary measurements of aerosol mass deposited on skin and hair were made during the series of tests; a typical result is given in Table 2. The result indicate that without exceeding the aerosol concentration at which coagulation might occur, a detectable mass of tracer can be measured on hair and skin.

Table 2. The result of a typical test (using 4 μm particles), carried out during the Ferslev series, to determine the detection limit for tracer measurement on skin and hair

Hair:	0.172 μg Dysprosium per gram
Skin:	20.65 μg Dysprosium per sq. metre
Initial aerosol concentration:	1.5 mg silica/ m^3

Publications

Byrne MA, Lange C, Goddard AJH and J Roed (1992). Indoor aerosol deposition studies using neutron activatable tracers. Journal of Aerosol Science 23, S1, 543-546.

Byrne, MA, Lange C, Goddard AJH and J Roed (1993). Indoor aerosol deposition measurements for exposure assessment calculations. Proceedings of the 6th International Conference of Indoor Air Quality and Climate (Helsinki), 3, 415-420.

Byrne MA, Goddard AJH, Lange C and J Roed (1993). Aerosol Deposition Measurements in a Test Chamber. Proceedings of the 7th Annual UK Aerosol Society Conference (Bristol).

Byrne MA, Goddard AJH, Lange C and J Roed (1993). Tracer Aerosol Deposition Experiments for Indoor Air Quality Assessment. Proceedings of a Conference on Allergy Problems in Buildings, London (a book resulting from the conference will shortly be published).

Head of project 5: Dr. J.Roed

Objectives for reporting for reporting period.

- Measure deposition constants in new houses, when available using both old and new particle sizes.

- Determine deposition velocities as function of surface orientation.

- Establish a protocol for measuring skin deposition.

Results.

In the spring of 1993 a new house became available in the village of Ferslev in Denmark. A two week experimental campaign was arranged with participation from Imperial College London. The visiting research assistant from Imperial College brought their new sub micron particle equipment, consisting of an Indium powder, a nebulizer and a alpha-source, used for neutralization of the particles. Table 1 shows the results of the 13 tests performed. The introduction of the sub micron particles was a success with reasonably straight decay lines and deposition velocities close to those predicted from earlier tests with larger particles. In general deposition velocities increased with particle size and degree of furnishing just as expected. Fig. 1 shows a decay curve for the sub micron particles. The obtained results were in good agreement with earlier results as can be seen on Fig.2. and fig.3.

Especially interesting was the comparison between the results obtained with the sub micron particles and the experiment by Roed and Cannell(1987, Radiation Protection Dosimetry). Roed and Cannell used stratospheric Be-7 as a tracer. Their conclusion was that there was no filtration by the building envelope and that the lower indoor concentration should be explained by an average(to all surfaces) deposition velocity of Be-7 labelled particles of 0.000071 m/s. This tracer was measured during the last CEC contract to have an AMAD of 0.7 to 1.0 μm. The sub micron particles used in

Ferslev had an AMAD of 0.7 μm and their average deposition velocity was found to be 0.000065 m/s. This close match is another indicator that the conclusion by Roed and Cannell was correct; i.e. that indoor deposition rather than filtration over the building envelope is the factor of importance when determining concentration of indoor air particulates of outdoor origin.

Deposition as a function of surface orientation.

During each test in Ferslev two filters where place on each surface; twelve in all for each experiment. The filters was put in the same positions each time to make the experiments more comparable. Average air concentration of tracer was determined from air filter samples taken in the middle of the room as in all our previous tests. By subsequent activation and analysis the amount of tracer on each surface filter was determined. The average concentration in the room was determined from the air samples and deposition velocities were calculated.

The results can be seen in table 2. The last row of the table shows the deposition calculated based on surface samples divided by that found by monitoring the decay of tracer in the air. The closeness of the number to unity should then be a indicator of how good an agreement exists between the two methods. It can be seen that good agreement is not found in tests 2 and 4. Deposition velocities to all the surfaces in these test are significantly larger than expected implying that an error in the estimate of the initial concentration might be the reason for the big difference. 11 and 13 did not show good agreement either but here it seems that the deposition velocity to the ceiling is the main source of error. The last six tests show a good agreement considering that the estimate on deposition constant is made based on samples from less than 0.02% of the surface of the room.

Skin deposition

During the last three tests in Ferslev samples where taken by wiping skin and cutting hair. The purpose of this was to verify

that these methods yields measurable and reasonable results. Results are reported by Imperial College.

Aims for the next year

During the next year the emphasis will be onto measuring deposition to specific surfaces; in particular skin, hair and clothes will be examined under 'real house conditions'. In the Risoe test chamber deposition surfaces as a function of orientation will be studied to verify/clarify some of the results from the Ferslev test house.

Modelling will be undertaken to evaluate the obtained results considering dose reduction factors. An estimate of kerma rates from indoor deposits compared to outdoor deposits will be worked out.

Achieved publications:

1) Byrne,M.A.; Lange,C.; Goddard,A.J.H(1992). and J.Roed. Indoor aerosol deposition studies using neutron activatable tracers. J. of Aerosol Science 23, S1, 543-546.

2) Byrne,M.A.; Lange,C.; Goddard,A.J.H(1993). and J.Roed. Indoor Aerosol Deposition Measurements for Exposure Assessment Calculations. Proceedings of the 6th International Conference of Indoor Air Quality and Climate (Helsinki), 3, 415-420.

3) Byrne,M.A.; Goddard,A.J.H.; Lange,C. and J.Roed(1993). Aerosol Deposition Measurements in a test chamber. Proceedings of the 7th Annual UK Aerosol Society Conference (Bristol).

4) Byrne,M.A.; Goddard,A.J.H.; Lange, C. and J.Roed(1993). Tracer Aerosol Deposition Measurements for Indoor Air Quality Assessment. Proceedings of a Conference on Allergy problems in Buildings, London(a book resulting from the conference will shortly be published).

no	par size µm	Decay con. h^{-1}	air ex. h^{-1}	Dep-vel. 10^{-4}m/s	Corr.	Furn. yes /no
1	2	0.583	0.052	0.917	0.79	no
2	3	0.67	0.113	0.960	0.97	no
3	10	1.856	0.122	2.995	0.98	no
4	4	1.198	0.077	1.937	0.93	no
5	0.7	0.451	0.050	0.693	0.94	no
6	3	1.052	0.067	1.700	0.99	no
7	10	1.821	0.046	3.07	0.97	no
8	0.7	0.308	0.046	0.531	0.84	no
9	10	2.002	0.128	3.24	0.97	Yes
10	3	1.327	0.028	2.25	0.98	yes
11	4	1.996	0.077	3.315	0.995	yes
12	0.7	0.41	0.077	0.575	0.92	yes
13	2	0.47	0.025	0.726	0.97	yes

Table 1. Review of results from the experiment in Ferslev.

no	par size µm	Vd Walls 10^{-4}m/s	Vd Floor 10^{-4}m/s	Vd Ceiling 10^{-4}m/s	Calc. Dep. Const.	Calc. DP/ DP
1	2	1.037	2.162	1.117	0.805	1.38
2	3	3.985	10.135	13.705	4.954	7.39
3	10	2.563	17.95	-	3.620	1.95
4	4	16.33	39.83	12.216	12.742	10.64
5	0.7	0.385	1.070	0.127	0.295	0.65
8	0.7	0.226	0.536	0.171	0.174	0.57
9	10	4.364	12.008	1.725	3.376	1.69
10	3	2.570	3.582	4.349	1.962	1.48
11	4	7.026	12.98	13.344	6.134	3.07
13	2	2.487	2.877	4.082	1.780	3.79

Table 2. Deposition velocities to specific surfaces.

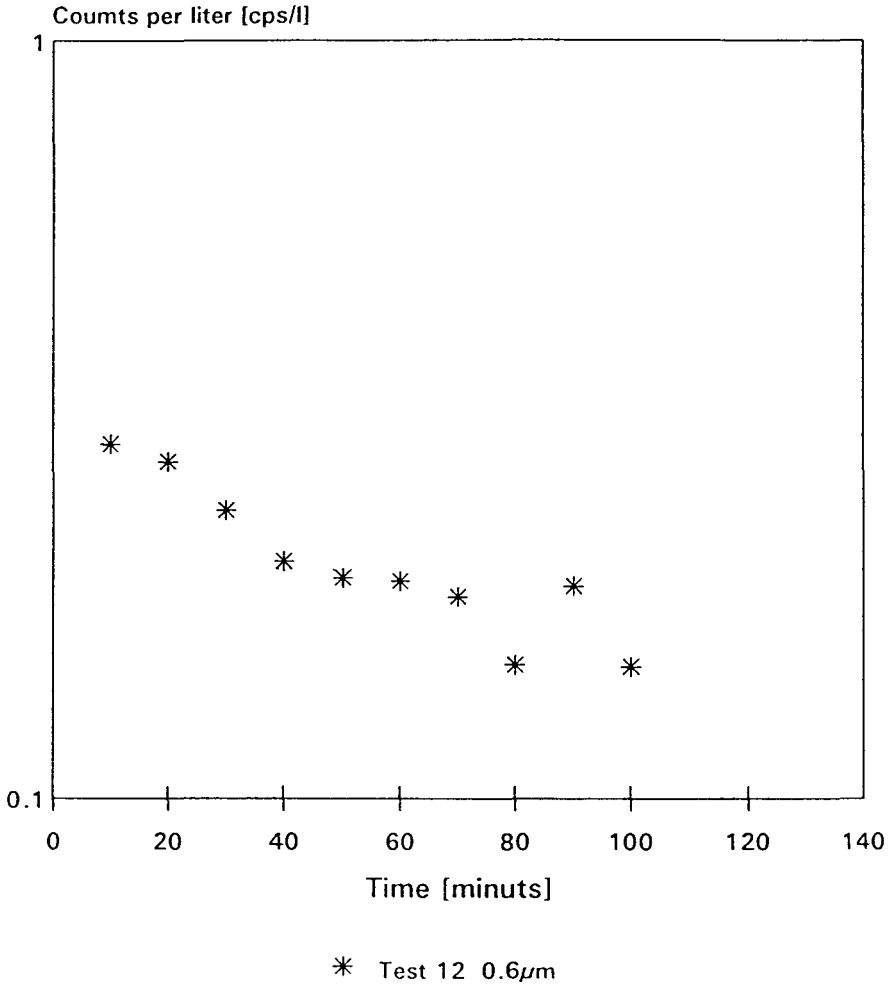


Figure 1. Decay curve for 0.7 μm Indium particles test 12. The correlation factor for this curve is 0.92 which smaller than earlier values. This is due to problems with our filter holders. They will be replace in future tests.

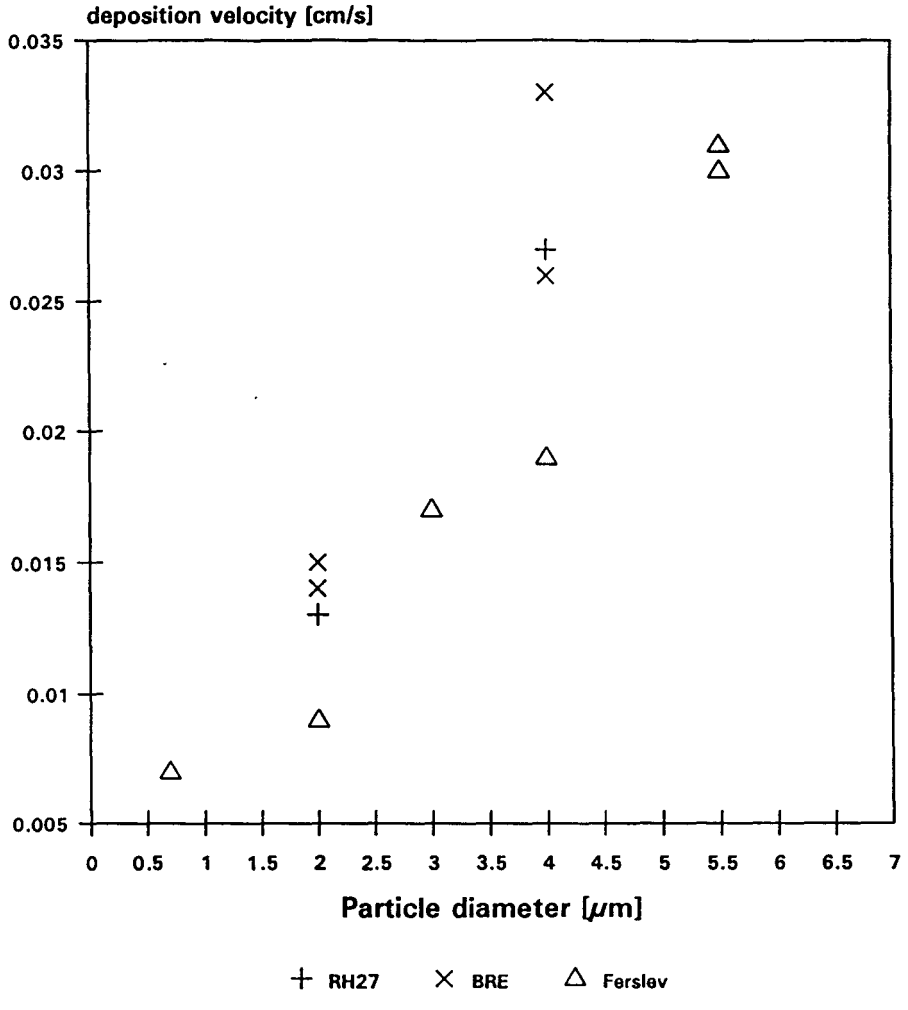


Figure 2. Average deposition velocities from three different unfurnished houses. The three different test series are: RH27, tests performed in Risoe Huse 27, Denmark autumn 1991; BRE, Building Research Establishment in Watford autumn 1991 and Ferslev in Denmark spring 1993.

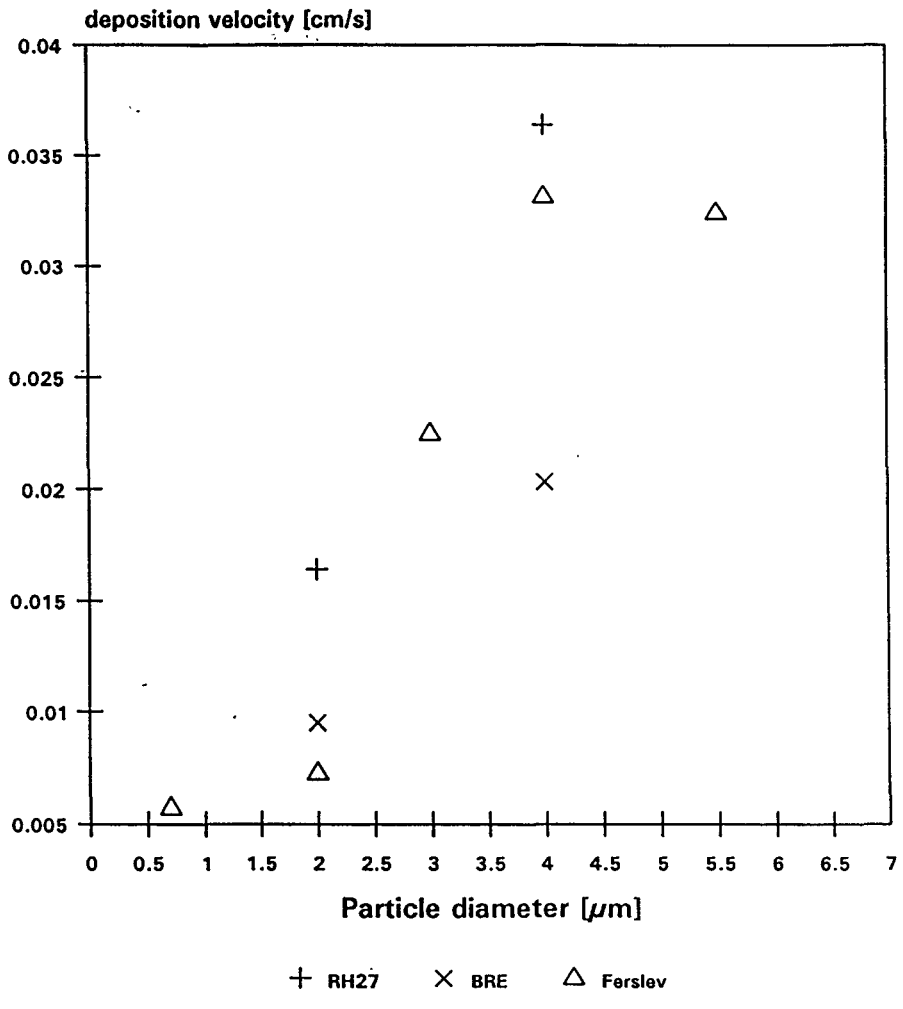


Figure 3. Average deposition velocities from three different furnished houses. The three different test series are: RH27, tests performed in Risoe Huse 27, Denmark autumn 1991; BRE, Building Research Establishment in Watford autumn 1991 and Ferslev in Denmark spring 1993.

Progress Report

Contract: FI3P-CT920044 Sector: C24

Title: Coordination of atmospheric dispersion activities for the real-time decision support system under development at KFK.

1)	Mikkelsen	Lab. Risø
2)	ApSimon	IMPCOL
4)	Desiato	ENEA
5)	Rasmussen	DMI
6)	Thykier-Nielsen	Lab. Risø
7)	Bartzis	NCSR "Demokritos"
8)	Massmeyer	GRS

I. Summary of Project Global Objectives and Achievements

The project goal is to coordinate the developments and construction of an atmospheric dispersion module for the joint European RODOS (Real-time On-line DecisiOn Support) system under implementation at KfK.

The project involves model development and integration with special consideration for user-friendly codes, user friendly interactive menus, interactive graphical systems. Highest priority is at present given to the dispersion and deposition modules applicable for the meso- and long range scales.

For the near-site range, the project is presently consolidating previously initiated phase-I activities, including:

Integrating meteorological pre-processor; On-line data transfer from numerical weather forecast (NWF) centers; Graphical user-interfaces; Hands-on training and exercise facilities; Experimental training and evaluation data bases; On-line data assimilation and back-fitting techniques for on-line radiological measurements.

Achievements during the period:
Local scale pre-processor flow, dispersion modules have been prepared for transfer and integration in RODOS. Models: ADREA DELTA DIPCOT have been documented for RODOS use and a provisional interface with regional NWF data have been established.

The 3-DRAW long range model have been improved for easier general use. Modules for convective storms and frontal systems have been complemented. Advances of integration with a Geographical Information System (GIS) have been investigated.

The operational weather forecast center at DMI have provided meteorological input data from three test periods: May 17. - 23. '93; July 8. - 21. '93 and Sep 8. - 21. '93.

Head of Project 1: Dr. Mikkelsen

II Objectives for the reporting period

- Coordinate RODOS atmospheric dispersion activities and to distribute appropriate model evaluation and test data for participants. Assist in model integration and model modifications to meet the requirements defined by the RODOS project.

- Provide detailed experimental diffusion data suitable for real-time modelling from full scale atmospheric diffusion experiments for various ranges and sites, ranges, atmospheric stabilities, source types and release scenarios.

- Establishment of a computer based "Reference and Training Experimental Data Base" for on-line training and hands-on application in the RODOS system.

- Improving the Risø mini-LIDAR systems for remote sensing of aerosol plumes at longer distances.

III Objectives for next period

Coordination and modelling activities:

- Data analysis and data distribution of flow and dispersion model evaluation experiments: GUARDO '90; BOREX '92; MADONA' 92.

- Data analysis and data distribution of new building wake experiment BORSSELE' 93.

- Model evaluation (with RODOS models PAD/LINCOM/MCF/RIMPUFF) based on experimental data from the GUARDO, BOREX MADONA and BORSSELE experiments.

Experimental oriented activities

- Continued data processing, quality assurance and corrections of experiments MADONA and BOREX' 92. Data analysis and documentation. Add experiments to the "RODOS uncertainty-knowledge and training data base".

- LIDAR II: Finalize construction and testing of 2. generation mini-LIDAR system suitable for two-dimensional plume scanning and diffusion measurements at longer ranges. Perform first tests with new LIDAR-II-system.

- Repeat full scale diffusion experiment over flat and homogeneous terrain with emphasis on longer scales (BOREX' 94). Compare LIDAR-I and LIDAR-II with tracer diffusion data.

- Compare HIRLAM-NWF data with meteorological observations of speed and direction from the 125 meter high Risø tower.

- Participate in the ETEX long range tracer experiment.

IV Progress achieved including publications

1) Established 3 regional weather data sets based on HIRLAM:

In order to establish and test an on-line coupling of the RODOS to a NWF weather forecast center it was decided to establish 3 test reference data sets data for distribution to participants.

Over the summer 1993, collaboration with the Danish Meteorological Institute have resulted in a huge amount of useful HIRLAM test data for the 100 meter level above sea level covering the entire of Europe, including a large part of Russia and the Atlantic Ocean.

To data, data were "logged" on mass storage devised at DMI during the following period: May 17. - 23. '93; July 8. - 21. '93 and Sep 8. - 21. '93 (incl.), and are pt. being transferred to the coordinator for distribution .

The period May 17-23 holds analyzed winds over Europe every 6 hr (4 times pr. day). The July and Sep. periods in addition forecasts at 3 hrs intervals up to + 48 hours.

The data storage requirements for one day amounts to some 22 Mbyte, so either selective data for local regions have to be identified, or new ways for transfer of huge data amounts between participation institutes have to be established (during next period). See also progress report no. 5 (DMI).

2) Full scale building wake diffusion experiment were performed from the nuclear power plant Borssele, NL:

With the purpose to study the influence of nearby building effects on-site atmospheric dispersion, a joint Risø/NERI full-scale diffusion experiment were successfully performed at the Borssele nuclear power plant near Vlissingen in Holland during Sep. '93.

Smoke were released from top of the generator building and also from the containment ventilation chimney. As during the previously BOREX' 92-experiments, joint Lidar and tracer techniques were used to measure the resulting downwind dispersion pattern.

Although data have not jet beeing analyzed, it was evident from both measurements and visual observations that building effects have tremendous effect on the on-site and near-range dispersion characteristics.
Results will be added to the RODOS experimental data base, once completed.

3) A new graphical visualization program ("S") have been provided for graphical presentation of model and experimental data:

Real-time handling and intercomparison of large amounts of vector fields (video-animation) resulting from real-time model and measurements has been facilitated by the construction of a (PC-based) GUI program suitable for RODOS models and experimental data. The programme also provides an easy-to-use and user-friendly training facility within the RODOS system. Demonstrations based on "S" were presented during the Schloss Elmau 1992 workshop (see refs Jørgensen et al. and Thykier-Nielsen et al.).

4) LIDAR-measurements and micro-meteorology data were provided to the MADONA flow and diffusion experimental data base:

The MADONA (Meteorology and Diffusion Over Non-uniform Areas) experiments took place Sep. 1992 at the famous atmospheric dispersion site at Porton Down in the UK.
As a joint venture, with participation from US Army, CBDE UK, DLR, Germany, Risø Denmark, FOA4 Sweden and more, we obtained detailed atmospheric dispersion measurements over rolling terrain on the near range scale supported by micro-meteorological mean and turbulence measurements for characterizing the atmospheric flow and stability.

Both ground and elevated puffs, tracer gas and ground level continuous smoke were released and measured by several Lidar systems, including Risø and DLR's mini-Lidar systems. With the Porton Down area being characterized by rolling and non-uniform terrain, extensive meteorological instrumentation (14 ten-meter high met towers, in addition to 2 on-line sonic anemometers) were recorded around the clock. Interesting non-stationary dispersion scenarios were here encountered during transition zones and best visualized by the "S" real-time graphics display programme, the above described tool for fast sequential presentations (movie) of 2-D fields of scalars (concentrations) and vectors (winds).

Extensive data reduction and preparation is now in progress in order for the MADONA trials to be analyzed and for part of it to be included in the RODOS training data base. All the participants MADONA data are merged at the GMGO military geophysical office in Traben-Trarbach, where also an UNIX based graphical display and presentation programs have been developed for visualization purposes. The RODOS project will advantageously could benefit from further collaboration with GMGO re. GUI's.

5) The first results from joint tracer Lidar elevated release experiment BOREX' 92 have been evaluated:

An elevated release diffusion experiment (BOREX '92) were conducted over flat terrain with smoke and tracer released from a 25 meter high meteorological tower.

Five daytime and one nighttime reference experiments were obtained during a one week campaign in July 1992, and lidar-measured plume statistics have been obtained at various downwind measurement points out to 1 km from the source point. The experiment were accompanied by simultaneous conducted tracer-gas diffusion experiments (SF6) by our danish colleagues at the NERI institute for mean-value determination and inter-calibration purposes.

Results analyzed to date have shown surprisingly good agreement with mean lidar data as compared to tracer-gas determined concentrations. This encouraging result enables us in the future to perform vertical plume scans with relative few ground based SF6- reference measurements. Also this data set is pt. being processed and analyzed.

6) A Joint DMI/Risø workshop on Nuclear Atmospheric Dispersion Forecasting were held in Copenhagen, Nov. '92:

Several meso- and long range nuclear dispersion models suitable for RODOS were presented and discussed, see also the progress report No. 5 by DMI.

7) Two new partners (Massmeyer/Martins - GRS, Köln) and (Sandor Déme - AERI, Budapest) were integrated in the project under the RODOS/ Atmospheric dispersion sub-programme:

GRS has already changed the input/output data structure of their mass-consistent flow model MCF in compliance with RODOS (see progress report No. 8), and AERI has contributed to the project with an effective algorithm for gamma dose assessments from individual (Gaussian) puffs.

8) Work has been initiated leading to a real-time wind observatory network based on existing (6-7) on-line met-towers:

The Meteorology section at Risø is continuously serving 6-7 on-line met-towers distributed around Denmark, including the Risø on-site 125 meter tall observatory mast. A new project has been started in collaboration with the Danish Emergency Management Agency in order to provide on-line wind data from these stations as a supplement and anchor point for the HIRLAM NWF data otherwise provided via to the Emergency Agency via computer networks.

This project enables an unique possibility for intercomparison of numerical and independent measured wind data.

9) Progress with construction of "LIDAR-II" for improved range and temporal resolution:

Progress with the new system has mainly been obtained with respect to new detector systems and a new computerized data acquisition system for fast data transfer and control. A new telescope has been purchased, and various new lasers have been investigated. Negotiations are pt. ongoing with Big Sky lasers. Inc. Montana, USA for purchase.

10) A Ph.D (Hans E. Jørgensen) was obtained, based on work relating to the activities within the Radiation Protection Research activities started back in 1989.

11) Within the Nordic NKS-BER framework, Denmark participated (on June 21. 1993 in a "real-time" long range dispersion and accident assessment exercise (organized by Ulf Tveten, IFE, Norway).

12) Finally, within the framework of NKS-BER, an attempt to harmonize presentation of dispersion model results has emerged (cf. the NORVIEW-standard suggested by the Finish Meteorological office).

V References

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Head of project 2: Dr. ApSimon

II. Objectives for the reporting period

One of the main objectives of the project, during this reporting period, has been to test the 3-DRAW (3-Dimensional Random Walk) model and make it easier for general use. This has included completion of the treatments in the model for convective storms and for the transport of material through complex meteorological conditions, such as cyclones and frontal systems.

In addition, having recognised the importance of Geographical Information Systems (GIS) as a means of communicating radiological data to decision makers, a further objective for this reporting period was to gain sufficient expertise with a commercially available GIS package.

A further objective was to begin work on integrating radiological measurements, taken in the field, with model results, so as to improve and revise further model predictions.

III. Progress achieved including publications

1. Consolidating the progress made in the previous contract:

i. Part of the present contract has been spent completing integration into the 3-DRAW model features that were developed during the course of the previous contract. To this end a new parametrization for convective storms has been successfully integrated into the model and tested. Since convective storms are likely to be particularly patchy with concentrated hot-spots it was felt useful to distinguish this type of rainfall from more uniform wet deposition likely in larger scale frontal systems. Consequently, 3-DRAW now treats the convective precipitation independently and can display it separately.
In addition, computer graphics (UNIRAS) have been developed that incorporate data from weather prediction models to display areas that may contain convective precipitation. This illustrates the spatial variability of wet deposition and will serve as a reminder to decision makers that care must be taken in these areas when planning emergency responses.

ii. New frontal system data has been supplied by the UK Met. Office and has been used in the further development, and testing, of a modified version of the 3-DRAW model (3-DFRONTS) that uses a variable time step instead of a time step of fixed duration. Application of both models to complex meteorological conditions showed that material released into the path of a cyclone produced significantly different dispersion patterns. However, the dispersion patterns produced by material that was tracked for several days through a relatively slow moving frontal system, were similar.

Some additional model testing is required to check that the transport of material in and around the warm conveyor belt is realistic, whilst some further development in the behaviour of the atmospheric boundary layer within frontal systems will be undertaken.

2. Progress achieved during this contract:

i. The integration of radiological measurements with the model results, to improve and revise further model predictions, is now under way. This draws on work carried out during the Chernobyl accident with the MESOS model as well as including the derivation of source terms. The work includes relatively simple techniques such as comparing time profiles of concentration at specified points. Techniques are also being developed that will allow the grouping and revising of particle positions, based on their time of release or their position, or for initiating new groups of particles in accordance with observations.

This work will be completed during the course of the next contract.

ii. We have been interested, for some time, in the use of Geographical Information Systems (GIS), either as a complement to the graphics manager GSY, developed at KfK, or as a fully integrated feature of a future version of RODOS. Preliminary studies with a commercially available GIS package (Arc/Info) has been undertaken. So far this work has been concerned mainly with geographical presentation of particle positions, contaminated areas, population centres and other features important to radiological protection. Future work will include the coupling of a dispersion model with a GIS in an attempt to perform interactive model nudging, and this work will represent a significant part of the following contract.

3. User friendliness and quality control:

i. In addition to the testing of specific aspects of the model as described above, some time has been spent removing the model's dependency on specific external packages such as the 'fine mesh' numerical weather prediction model. It is hoped that this will make the model more flexible and easier to integrate into the final RODOS package.

ii. As each new feature has been integrated into the model, a large proportion of time has been spent on quality assurance. Clearly quality assurance will continue in the next contract as will improved user friendliness for the models.

Publications:

Lowles I.M., H.M. ApSimon and Wilson J.J.N., *The validation and uncertainties of real-time modelling of atmospheric dispersion over medium and long-range distances.* Radiation Protection Dosimetry (in press).

Head of project 4: Dr. Desiato

II Objectives for the reporting period

The objective of the activity is in the context of providing and assisting the implementation of usable modules into the Analysing Subsystem (ASY) of RODOS. ENEA-DISP is involved in the development and application of software modules for pre-processing the meteorological data in order to provide the atmospheric dispersion models with the necessary input in real-time. A number of routines for estimating with different methods the model input parameters have been developed. A software package (PAD: Pre-processor for Atmospheric Dispersion models) for using and testing the routines has been developed in a DEC-UNIX environment. With the purpose of including the pre-processing routines into a centralized, computerized emergency response system such as RODOS, a functional scheme for the selection in real-time of the appropriate meteorological observations and methods has been designed.

For the next period, the pre-processing capabilities will be extended to the preparation of turbulence parameters needed by three-dimensional dispersion models. In particular, the attention will be focused on incorporating routines for deriving the vertical pofiles of the wind fluctuation components and of the Lagrangian time scales into the ARCO model (Atmospheric Release in COmplex terrain). ARCO is a particle model for short/medium range dispersion in complex topography areas, and is suitable to be coupled with a diagnostic mass-consistent wind field model such as MCF (from GRS Koln).

III Progress achieved

The meteorological pre-processor includes several routines for estimating Monin-Obukhov length, friction velocity, sensible heat flux, mixing height, wind speed profile, atmospheric stability, and turbulence parameters. Each routine has been tested for a wide range of values of primary meteorological observations, using the PAD package implemented on a RISC DEC Station 5200 in ULTRIX Operative System. For each parameter the input can be edited throuh guided masks and the output can be displayed in the form of tables or graphics (time diagrams).

A functional scheme proposed for linking the meteorological preprocessor with the on-line meteorological data base and the atmospheric dispersion models included into a centralized, computerized emergency response system such as RODOS has been designed. This scheme actually corresponds for many aspects to what has been recently developed and is presently operational into the ARIES-I system at ENEA-DISP.

The starting point is that n_v parameters $\mathbf{v_i}$ $(i=1,n_v)$ are eventually needed by an atmospheric dispersion model in the time interval $t_0<t<t_f$, which is the model simulation time. The time interval can cover the "past", or the "future", or both, depending on the content of the on-line meteorological data base, whether it includes observations only or forecasted data too. The analisys is limited for the moment at meteorological data at single 'points', or stations, as are generally used for short-range dispersion modelling. A parameter can be evaluated using different methods $\mathbf{m_{ij}}$ $(j=1,n_m(i))$, each based on a set of n_p primary meteorological observations $\mathbf{p_{ijk}}$ $(k=1,n_p(i,j))$.

The procedure is based on three statements. The first statement says that the method for deriving one parameter must be the same for the whole simulation period, namely it is not convenient to use different methods in order to avoid dishomogeneities in the evaluation of a parameter during the same dispersion episode. The second statement says that a "hierarchy" of methods can be defined "a priori", on the base of their physical soundness and on validation studies available. The third statement says that a "space" representativeness, $\mathbf{d_{ijk}}$, and a "time" representativeness, τ_{ijk}, can be assigned a priori to each primary meteorological data $\mathbf{p_{ijk}}$. Let's consider, as an example, that the index i correspond to the stability category, the index j to the "radiation index" method and the index k to the cloud cover. Two possible values for $\mathbf{d_{ijk}}$ and τ_{ijk} are 50 km and 90 minutes, i.e. we consider an observation of cloud cover valid in a range of 50 km around the reporting station and for a time interval within -90 and +90 minutes by the reporting time. In this way a "validity table" of values of $\mathbf{d_{ijk}}$ and τ_{ijk} could be compiled and eventually modified "ad hoc" in case of a particular terrain or meterological condition.

Based on the above statements, the following procedure (fig. 1) is suggested. For each model input parameter v_i the first method of evaluation is considered, which requires $n_p(i,1)$ primary observations that must satisfy the $\mathbf{d_{i1k}}$ and $\mathbf{t_{i1k}}$ conditions, for $k=1,n_p(i,1)$. From the on-line meteorological data base the data of n_s meteorological stations s_l ($l=1,n_s$) located within a range $\mathbf{r<d_{i1k}}$ from the release point are extracted, and the τ_{i1k} condition is checked for the data of a single station at a time, starting from the station nearest to the source. If the condition is satisfied, i.e. if the longest time interval between two primary observations of at least one station is less than $2\tau_{i1k}$, the $\mathbf{p_{i1k}(t)}$ values are stored. If no stations satisfy the τ_{i1k} condition, the observations of different stations are merged, so that a new time series is obtained and undergoes the time validity check. If now the τ_{i1k} condition is satisfied, the $\mathbf{p_{i1k}(t)}$ are stored, otherwise a new method (j=2) which requires different $\mathbf{p_{i2k}}$ ($k=1,n_p(1,2)$) parameters is tried.

When all the $n_p(i,j)$ observations required by a method j are obtained, they are passed to the PAD routine for the calculation of the parameter $\mathbf{v_i(t)}$, and the procedure is iterated for all the n_i model input parameters. If no method exists which satisfies the validity conditions for all the necessary primary observations, the model run is impossible. An extra possibility can be given by considering a "relaxed" validity table, with values of $\mathbf{d_{ijk}}$ and $\mathbf{t_{ijk}}$ less restrictive then the previous ones, and starting again the procedure from the first method.

With the procedure described above, the situation of meteorological data coming from a single station is preferred, and data coming from different stations, provided they are still representative in space of the release point, are considered only if the data from a single station don't cover with a sufficient time resolution the time interval of the model simulation.

This procedure is quite general, in the sense that no assumptions are made on the type and the number of meteorological data and stations available on the on-line data base, nor on the dispersion model that must be linked with the preprocessor. Perhaps it could be simplified if the characteristics of these components of the system were fixed.

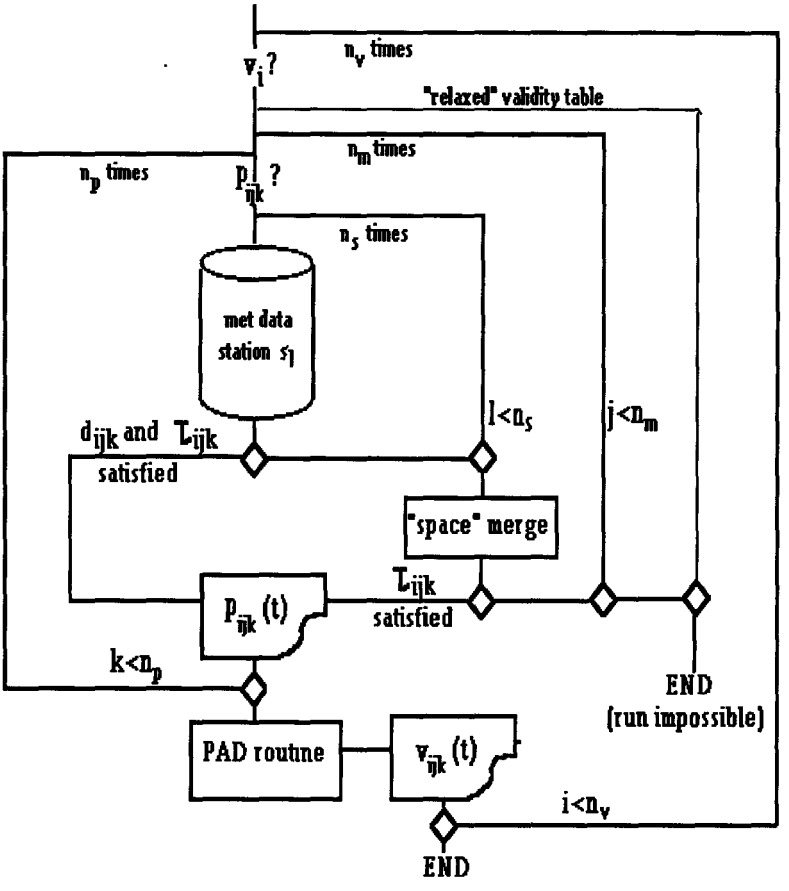
v_i ?
n_v times
"relaxed" validity table
n_m times
n_p times
p_{ijk} ?
n_s times
met data station s_l
d_{ijk} and T_{ijk} satisfied
$l<n_s$
$j<n_m$
"space" merge
p_{ijk} (t)
T_{ijk} satisfied
$k<n_p$
END (run impossible)
PAD routine
v_{ijk} (t)
$i<n_v$
END

Fig. 1

Head of project 5: Mr. Rasmussen

II. Objectives for the reporting period

To contribute to the development and validation of a real-time decision-support system using meteorological forecast data from the Danish HIgh-Resolution Limited-Area Model (DMI-HIRLAM) as input for the atmospheric dispersion models for dose calculations on local and regional scale.

In particular to:

- Examine which of the many parameters from DMI-HIRLAM can be used with advantage in dispersion models. Validation studies will mainly be based on new (actual) data
- Investigate the possibility of transferring large quantities of meteorological data on-line via electronic networks in collaboration with Risø and KfK

II. Objectives for the next reporting period

In general, to assist and to provide further forecast and analysed data from the numerical weather-prediction model DMI-HIRLAM for case studies with RODOS.

In particular to:

- Provide, in collaboration with Risø, single grid-point meteorology (from HIRLAM using either Kalman filtering technique or from LINCOM (WASP version) simulating a single weather station for local-scale forecast
- Provide the RODOS project with DMI-HIRLAM wind-field data over Europe for the ETEX experimental period for post-analysis
- Provide DMI-HIRLAM data (multiple layers) for the Imperial College study with the 3-DRAW model
- Further investigate fast file-transfer methods of large data quantities via electronic networks (Internet (using ftp), ISDN...)
- Investigate possible RODOS participation under the European agreement for open commercial provision of meteorological services (ECOMET) throughout Europe

III. Progress achieved including publications

The resolution of the DMI-HIRLAM model has been improved considerably. The model now utilises 31 vertical layers (previously 16), and the model is run operationally in two versions: GRV with a horizontal resolution of about 46 km and forecast length +48 hours is covering all of Europe, inclusive a large part of Russia, and the northern part of the Atlantic Ocean. DKV has a higher horizontal resolution of about 23 km and a forecast length of +36 hours, but does not cover the southern part of the EC. It is contemplated to run DMI-HIRLAM with an even higher horizontal resolution of about 5 km for a smaller area.

Objective verification [1] has been undertaken continuously, directly comparing forecast fields with observations from roughly 150 synop stations and 70 radio-sonde stations (selected by the European Working Group for Limited-Area Modeling). The results show that the higher resolution of DMI-HIRLAM has improved the forecast, and comparisons with UK-LAM (United Kingdom Limited-Area Model) show that DMI-HIRLAM generally has a better performance for the wind forecast. For other parameters, such as temperature and msl pressure, the verification results are quite similar for the two models.

An interface to the operational database containing the output from DMI-HIRLAM has been developed (and is continuously being improved/altered). This interface is capable of extracting relevant data for the RODOS project for a given geographical sub-area. Thus, GRV data for the period May 17, 00 UTC to May 22, 00 UTC (1993) were extracted for an area covering all countries of the EC and large parts of Russia (in total 6988 grid points in the horisontal). It was chosen to use data for model level 30 (the second-lowest level, about 100 meters above ground), and to extract the following parameters: wind speed and direction, 3-hour accumulated precipitation on the ground, height above ground for level 30, and the orography. These data amounts in size to about 8 Mb. At the time of writing, a similar extraction of selected data from the GRV model is performed.

Attempts have been made to transfer meteorological data (2 Mb) through the Internet by using ftp—with limited success, however. Apparently, there is a need to use a network with a higher capacity. ISDN is a potential candidate.

The height of the atmospheric boundary layer, which is of great importance in dispersion models, is not calculated by the HIRLAM system. However, it is contemplated to incorporate the scheme by A.A.M. Holzlag *et al.* [2] in the post-processing of DMI-HIRLAM.

At DMI we have developed a Lagrangian dispersion model for nuclear-emergency purposes, cf. Ref. [3]. The calculations are based upon analyzed and forecast meteorological fields (DMI-HIRLAM or ECMWF). The model simulates release of material in the atmosphere from one or several simultaneous emission points which may be moving. In order to describe well an emission of material in the atmosphere from a source extending a large land area, it may be important to use more than one emission point, e.g. in case of burning oil wells as the ones in Kuwait. In case of emergency situations such as a radioactive release following a shipwreck involving a nuclear-power driven ship or submarine, it is relevant to describe moving emission points. Furthermore, the dispersion program may be used in attempting to locate potential emission sites in situations where no information about accidental releases of hazardous material in the atmosphere has been given. Such use of the program involves integration backwards in time. The results of a run of the dispersion program may be presented in the form of trajectories for the released air parcels, or in the form of 'snapshots' showing the positions of the released parcels at a given time.

DMI has purchased the Lagrangian long-distance transport nuclear-accident response model NAME [4] from U.K. Met. Office. However, the model has not yet been implemented at DMI. The RIMPUFF model [5] has been obtained from Risø and is presently being implemented at DMI.

In November 1992, DMI and Risø (co-sponsored by NKS) arranged a workshop entitled "Workshop on Nuclear Atmospheric Dispersion Forecasting". The workshop with participation from CEC-RODOS (all except ENEA were represented) focused on long-range transport. Invited lecturers included Drs. F.B. Smith and H. ApSimon, Imperial College, UK, Dr. R.H. Maryon, U.K. Met. Office, and Mr. G.H.L. Verver, KNMI, the Netherlands. Regarding the use of output from numerical weather-prediction models (DMI-HIRLAM say), the space- and time-resolution requirements for adequate dispersion calculations were discussed at the workshop. Also the need for uncertainty assessment of dispersion calculations using either forecast or analyzed fields was emphasised.

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[5] S. Thykier-Nielsen, T. Mikkelsen, S.E. Larsen, I. Troen, A.F. de Baas, R. Kamada, C. Skupniewicz, and G. Schacher, in *Air Pollution Modeling and Its Application VII,* A Model for Accidental Releases in Complex Terrain. Ed. H. van Dop (Plenum Publ. Corp., 1989) 65-76

Head of Project 6: Dr. Søren Thykier-Nielsen

II Objectives for the reporting period

Inclusion of gamma dose modelling from puff's in RIMPUFF.
Interfacing with HIRLAM numerical weather forecast (NWF) model in the emergency preparence system.
Interfacing atmospheric dispersion models with other modules (MCF, PAD etc.) in the RODOS emergency preparence system.
Interfacing with the ENEA PAD pre-processors for the RIMPUFF/LINCOM system.
Back-fitting of modelled flow fields to wind observations.
Creation of a new flexible user-interface for the RIMPUFF/LINCOM system.
Flow modelling for unstable and stable meteorological situations.
Visualization system for display of temporal and spatial variations in meteorological data and in calculated concentration fields.

III Objectives for next period

Implementation and testing of new models for plume rise, building wake and dry deposition RIMPUFF.
Development of a gamma dose model for asymmetrical puffs.
Development and testing of the interface with regional scale models in the emergency preparence system.
Implementation of precipitation fields in RIMPUFF.
Implementation of 3-D wind fields in RIMPUFF.
Development of the interface with pre-processors for the RIMPUFF/LINCOM and the RIMPUFF/MCF systems.
Testing and further development of the user-friendly interactive version of RIMPUFF, based on the ARGOS-NT system.
Consolidation of the interface between RIMPUFF and RODOS.
Optimization of the computer codes for RIMPUFF and LINCOM.
Model evaluation.

IV Progress achieved including publications

IV.1 Introduction

During the contract period high priority has been given to the interfacing of RIMPUFF with the RODOS system. Work has concentrated on topics which are important in connection with the local scale atmospheric dispersion modelling in the emergency response system. An interface between RIMPUFF and the regional scale wind prognosis model HIRLAM has been created in close cooperation with DMI. Emphasis has also been placed on visualization of model results and other aspects of user training.

IV.2 Interfacing with RODOS

All communication between the operating system in RODOS and the atmospheric

dispersion module should take place through COMMON statements. Therefore a special version of RIMPUFF is being created and transferred to KfK. This version is without write and read statements. Further the updated modules for calculations of gamma-doses are included in this version.

IV.3 Gamma dose modelling

In cooperation with CRIP, Budapest, development of fast subroutines for the calculation of gamma doses from airborne and deposited radioactive isotopes, released to the atmosphere from a nuclear power plant. The first versions of these subroutines have been included in RIMPUFF and tested for typical releases of radioactivity. Work has started on the second step that includes gamma-doses from asymmetrical puffs.

IV.4 Flow modelling

For complex terrain fluid-equation based wind field calculations are normally based on a single upwind and terrain unperturbated observation. However, for neutral conditions, a procedure has been set up to calculate a wind field, which gives the best fit to a network of simultaneous observations at multiple stations. This fitting procedure has been implemented in RIMPUFF.

In cooperation with GRS, Köln, RIMPUFF has been interfaced with the mass-consistent flowmodel MCF. This version of RIMPUFF will also be connected to RODOS, see project 8.

The improvements of LINCOM continue by including non-neutral temperature forcing, so it becomes able to calculate gravity flows such as up-valley breeze and down valley drainage circulations.

IV.5 Visualization of model results

New computer programs displaying time series of calculated and measured dispersion data has been developed. Most important is the program called S, designed as a tool for time-series representation of a large number of data fields. Several kinds of data (vectors, scalars etc.) can be analysed. The program is applied in the analysis of the 1990 Guardo and the 1992 MADONA trials.

In cooperation with the Danish Emergency Management Agency, a version of RIMPUFF is being created for running under the WINDOWS-NT operating system. This new system includes simple menu-driven information displays, showing results, and drawing attention to important features or anomalies. In this project substantial effort is assigned to methods of assessing and interpretation of the model results, and to their communication with the user through user friendly graphics and display systems. A preliminary version of this system will be available in the beginning of 1994.

IV.6 Model evaluation

A series of 15 full-scale dispersion experiments from the 1990 Guardo trials, carried out over complex terrain in Northern Spain are being analysed. Actual wind and turbulence measurements taken during the experiment are used as input data for a series of simulations made with Risø's combined flow and diffusion model (LINCOM/RIMPUFF). Considerable effort is devoted to the testing of the improved features for taking in to account wind shear and plume rise in RIMPUFF.

In September 1992 the 3-week lasting MADONA (Meteorology And Diffusion Over Non-uniform Area) trials took place at Porton Down, UK. Here both ground and elevated puffs, tracer gas and ground level continuous smoke were released and measured by several Lidar systems. With the Porton Down area being characterized by rolling and non-uniform terrain, extensive meteorological measurements were recorded around the clock. Evaluation of this experiment has started using RIMPUFF and LINCOM.

The experimental evaluation of flow field and dispersion modelling, using data from the complex terrain SIESTA experiment (SF6 International Experiment in STagnant Air) has continued. The possible improvements of the simulations using multi-level precalculated flow fields are being investigated.

A system has been created for transferring regional scale flow and precipitation field data from the HIRLAM flow model to RIMPUFF. This system is tested in a project concerning the assessment of the possible consequences in Denmark from radioactivity releases from nuclear power plants in the Baltic countries.

Dose pattern from 7 day release starting on July 9., 1993

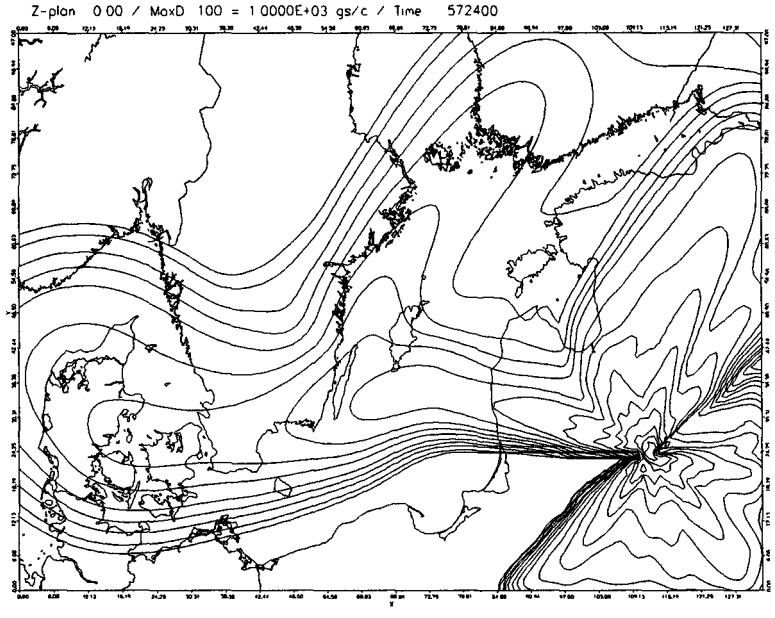

Figure 1. Dose pattern from a fictive 7 day release from a nuclear power plant in the eastern part of the Baltic

IV.7 Preprocessing of input data

Preprocessing of meteorological data for dispersion models has been studied in cooperation with ENEA (*Project no. 7*). The implementations in RIMPUFF of these pre-processing subroutines are being consolidated.

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Head of project 7: Dr. Bartzis

II. Objectives for the reporting period

Integration of the available modules/codes (i.e. ADREA, DELTA, DIPCOT) into the RODOS system including links with regional forecasts.
Demonstration of modules reliability and evaluation of the uncertainties based on well documented field data.
Important aspects of deposition such as dry deposition over seas and "radioactivity load" in water accumulated areas have to be looked more systematically.

III. Progress achieved including publications

The present activities can be divided in the following tasks.

1.Module integration into RODOS system

The RODOS system prototype No 1 is scheduled to be installed in "Demokritos" during the second half of 1993. Scientists from NCSR "Demokritos" have been participated in the introductory course organized by KfK in June 1993. The major effort has been concentrated on one hand linking the available modules with the regional forecasting data supposed to be available into the RODOS system and on the other hand to the production of ADREA and DELTA Modules documentation for RODOS users /1,2,3/. A diagnostic model has been built into our code system based on regional forecasting data to drive the dispersion modules. A new capability to the ADREA module (wind field + dispersion) is under development that allows the model to be nested within the regional forecasting data. The data used for development and testing are the ones given by ECWMF model via the Greek Meteorological Service in a 150x150km grid over Europe. Work will be started on HIRLAM data as soon as those data will become available.

2. Module evaluation

Within the framework of the DELTA-ADREA verification studies, interpretation of experimental data has been carried out and corresponding model calculations have been performed in three main cases of available observational data.

The first application concerns the local, thermally driven flows, generated at mountainous areas (e.g. anabatic/katabatic wind circulations) and utilizes the SF_6 release data and the relevant meteorological data-set of the experiment conducted in the region surrounding the Rocky Flats Plutonium Plant (Colorado, USA), west of the Rocky Mountains foot. The above terrain is representative of a particularly complex one, containing high mountain ridges and narrow canyons. The model simulated the February 4 case and produced, at several locations, successful comparisons of the obtained results and the observations, concerning a) the profiles of the horizontal wind components and air temperature, b) the time series of the screen hight temperature and surface net irradiance and c) concentration distribution /4/. Preliminary model applications have been also made, incorporating the "abrupt" time variation of the solar radiation input to the surface, due to the shading by other inclined surfaces during some period of the daylight (e.g. slopes within narrow canyons). The obtained results indicated that the above effect is rather significant and will constitute a subject of further development.

The second and the third application concern the examination of the daytime thermally driven flows, created at seaside regions (i.e. sea breeze circulations). The model was applied and evaluated, by comparing the obtained results with measurements, in two cases of field data referring to the surrounded by sea Attika district (Greater Athens area, Greece). In the first case the predictions of wind components and air temperature profiles and surface time series were satisfactorily compared with corresponding observations, at several locations of the greater area /5,6,7/. The second application produced also a satisfactory qualitative and quantitative simulation of the sea breeze circulation along the eastern coast of Attika and the results were successfully compared with tethersonde and surface data of the wind speed and direction and the diurnal and temporal variation of the air temperature and mixing ratio /8, 9, 10/.

3. Dry deposition over seas

A literature review was performed in order to determine a dry deposition model appropriate for the calculation of dry deposition at the air-sea interface /11/. The model that was implemented in the ADREA code is described in /2/.

4. Radioactivity load over water accumulated areas

As far as the radioactive pollutants conveyed by rain water are concerned, an adequate submodule named HYDRO has been created within the DELTA module /3/. Precipitation episodes of variable duration occurring in various locations of the topography considered may be taken into account. Related runoff stream
channels network and radioactivity accumulation areas are determined on the modelized ground surface assumed impermeable. An illustrative case related to the watershed at the Marathon Lake region near Athens is currently under treatment.

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Head of project 8: Dr. Maßmeyer

ii. Objectives for the reporting period

GRS contributes to the atmospheric dispersion activities within the real time decision support system RODOS in the context of providing an already existing mass consistent flow model (MCF) to the decision support system and to assist the implementation.

In order to enable the coupling of MCF to the dispersion model RIMPUFF interfaces concerning data transfer between the two models have to be specified. These interfaces should meet the criteria of OSY (the data organisation and transfer supervisor of the RODOS system) which have to be designed at the research center of Karlsruhe (KfK-INR). Hence, local and global parameters of MCF have to be specified and the input-output structure has to be modified. The updated version of MCF has to be distributed to Risoe and KfK for additional testing.

For a case study the coupling of MCF and RIMPUFF has to be demonstrated.

Objectives for the next reporting period:

The updated and modified PC-based versions of MCF and RIMPUFF have to be implemented in the RODOS prototype version of KfK. Test calculations concerning data transfer have to be carried out. As soon as meteorological data become available for use by the contractors, MCF will be adapted/modified to enable forecast calculations (by the end of 1993). At the beginning of 1994, the participants will meet to come to an agreement on an internal local scale model chain intercomparison exercise. As a documentation of the updated version of MCF a users manual will be prepared.

iii. Progress achieved including publications

Following the time schedule defined within the minutes of the RODOS coordination meeting on meteorology and atmospheric dispersion (24./25.2.1993 at KfK) a description of the global data of MCF has been sent to KfK.

Guided by a KfK-supplied example description of the procedure for integrating an external program in the RODOS system the modular structure of MCF has been modified.

In order to demonstrate the coupling of MCF and RIMPUFF a comparison of calculated tracer concentrations with measured data has been carried out. This case study is based on data which have been sampled in the vicinity of the research center of Jülich (KFA) in 1988.

The region around Jülich is characterized by flat terrain with predominant agricultural land use, scattered villages and forest areas. Since 1979 due to coal mining activities a waste hill (Sophienhöhe) and an open cast area has been built up. The maximum elevation of the Sophienhöhe is 190 m above and the minimum terrain height inside the open cast area is 160 m below the flat area. During the last 8 years several tracer experiments have have been carried out to analyse the influence of the Sophienhöhe and the open cast area on atmospheric dispersion from sources inside the KFA. A platform (50 m above ground level) of the meteorological tower of the KFA has been used as the source point. Several experiments under different meteorological conditions have been conducted, emitting SF6 at a constant rate during periods of 60 to 90 minutes duration. Up to source distances of 10 km airborne SF6 concentrations leewards of the KFA and in the area of the Sophienhöhe have been measured at several locations.

In order to demonstrate the coupling between MCF and RIMPUFF the experiment carried out on August 31st, 1988 has been chosen. The meteorological situation is characterized by an instationary early evening transition from neutral to stable atmospheric stratification. During this 1.5 hour period the wind direction has turned by 20 degrees. The tracer data show a marked influence of the Sophienhöhe on the flow field resulting in a curved centerline (around the hill) of the SF6 plume.

The calculation of this tracer plume with the coupled version of MCF and RIMPUFF is done in two steps:

- Wind data (10 min. mean values) measured during 1.5 hours and at 6 different locations between KFA and Sophienhöhe have been used as an input to drive the diagnostic model MCF. The output comprised an instationary wind field (changing every 10 minutes) and a mean wind field for the whole experimental period. As an example and to illustrate the effect of the Sophienhöhe on the near surface flow field, the averaged data for the layer 15 m above ground level are shown in figure 1. The arrows point into the direction of tracer transport, the length is proportional to wind speed. The flow is characterized by a south westerly component in the region of the KFA which changes in a southerly component west of the Sophienhöhe. The flow accelerates at the top of the Sophienhöhe and decelerates in the open cast area.

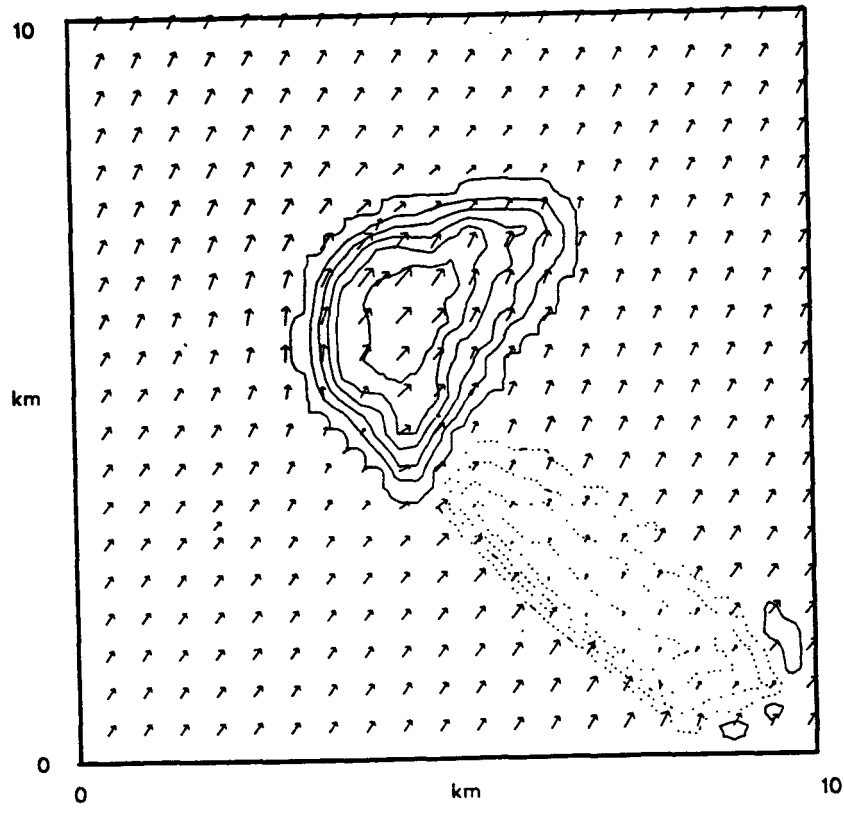
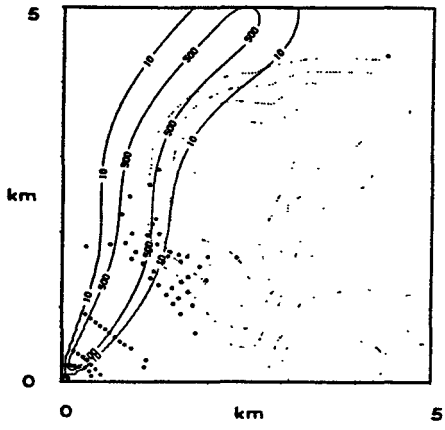


Figure 1: Wind field in the region of the Sophienhöhe (continuous isolines) and the open cast area (dashed isolines). The prevailing wind has a south-westerly component.

- The RIMPUFF-calculation of SF6 concentrations leewards the KFA is based on the 3-D wind field of MCF and additional information concerning turbulence parameters. The results of three different coupling scenarios are shown in figure 2:
  - averaged (1.5 h) MCF wind field for the experimental period and turbulence parameters for RIMPUFF based on Pasquill diffusion category F (upper figure)
  - instationary MCF wind data (for 10 min. periods) and turbulence parameters based on Pasquill diffusion category F (figure in the center)
  - instationary MCF wind data and turbulence parameters deduced from time dependent measurements of a vector wind vane (lower figure).

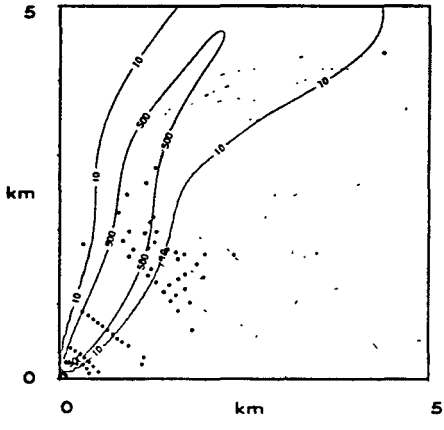
As can be seen the prevailing stable stratification leads to a tracer plume around the Sophienhöhe. This is in agreement with the measured SF6 data.



stationary flow field;

turbulence parameters according to

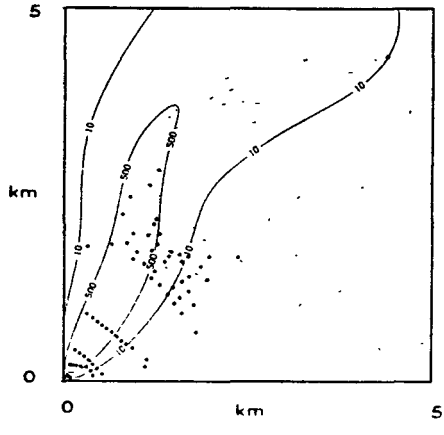
Pasquill diffusion category F



instationary flow field;

turbulence parameters according to

Pasquill category F



instationary flow field;

turbulence parameters according to time

dependent data of a vector vane

Figure 2: Time integrated near surface concentrations of SF6 (in 10^{-5} gs/m^{-3}) for the dispersion experiment carried out at the research center of Jülich on August 31st, 1988.

Progress Report

Contract: **FI3P-CT920057** **Sector: C24**

Title: Methodology for evaluating the radiological consequences of radioactive effluent released in accidents - the MARIA project.

1)	Jones	NRPB
2)	Ehrhardt	KFK
3)	Alonso	Univ. Madrid - Politécnica
4)	Van der Steen	N.V. KEMA

I. Summary of Project Global Objectives and Achievements

The overall objectives of the project are:

i) The maintenance, support and development of the COSYMA system. This includes modelling and data improvements and updates to both the system itself and the supporting documentation, and continual adaptation of the system to the needs of users.

ii) The further development of a PC version of the COSYMA system, to allow non-expert users to undertake ACA studies without access to large computer resources. A feature of this system is an interactive interface. It is intended that the software for the PC system, like the full COSYMA system, will be available to EC organisations through the CEC.

iii) Participation of the COSYMA system in the CEC/NEA ACA code intercomparison exercise, and documentation of the results.

iv) Further work on the development of techniques for assessing economic impact, including impact beyond the areas affected by countermeasures, the incorporation into the COSYMA system of an alternative economics model, and site-specific features of economics modelling.

The principal achievements during the period of the contract have been:

i) the preparation of a further version of the COSYMA system, to be released in 1993,

ii) the preparation and release of the first version of PC COSYMA,

iii) participation of COSYMA in the NEA/CEC ACA code comparison, and the formation of a COSYMA users group,

iv) design of a methodology for assessing the economic impact of accidents both in the areas affected by countermeasures and beyond them.

These are described in more detail in the later sections of this progress report.

Head of project 1: Dr J A Jones

II. Objectives for the reporting period

i) To assist KfK in the maintenance, support and development of the COSYMA system, primarily in the area of data improvements and updates to the system and the supporting documentation.

ii) To take the lead, with assistance from KfK, in the further development of the PC version of the COSYMA system, with the aim of producing a first version for release early in 1993.

iii) As part of the CEC/NEA ACA code intercomparison exercise, to participate together with KfK, KEMA and other EC organisations, in the running of the COSYMA code, and the documentation of the results.

The objectives for the next reporting period are:

i) To continue to assist KfK in the maintenance, support and development of the COSYMA system.

ii) To further develop PC COSYMA, with assistance from KfK, leading to a revised version, with graphical display features and improvements and enhancements to other parts of the system, for release in 1994.

iii) To undertake a review, with assistance from the other participants in the project, of the current position of probabilistic ACA modelling and codes, with a view to identifying the needs and priorities for future research.

III. Progress achieved including publications

1 Work in Support of Mainframe COSYMA

The first version of COSYMA, developed by KfK and NRPB, was released in 1991. Since then a number of refinements to models and data have meant that COSYMA can be updated. A revised version of COSYMA, designated 93/1, has been prepared with KfK for distribution later this year. Within the reporting period a number of discussions on the models and data bases to be included in the version 93/1 have been held with KfK. Some of these are described in more detail in the KfK section of this progress report. The work at NRPB to generate new data files for inclusion in version 93/1 is described in sections 1.1 and 1.2 below.

1.1 Incorporation of the Results of EXPURT into COSYMA

The NRPB model EXPURT predicts external γ doses and dose rates following deposition of radioactive material. The model considers the amount of material deposited on different surfaces in residential areas, the movement of material between these surfaces and into the soil

column, and the dose from material on the different surfaces, and so is more detailed and realistic for urban areas than the more simple models routinely used in accident consequence codes. A study has been carried out to examine ways in which the results of EXPURT could be incorporated in the COSYMA code. Runs of COSYMA and the NRPB MARC programs were carried out for a source term containing only a few nuclides. These were selected to include those giving important contributions to the deposited γ dose. Some of the runs used the COSYMA deposited γ dose libraries while the other used libraries calculated with EXPURT for different environments representing different levels of urbanisation (ie. inner city, residential and rural), and for deposition in wet and dry conditions. A comparison of the predictions suggested that using an average of the EXPURT files for the three environments would give results in reasonable agreement with those obtained by explicit averaging over the different types of environment.

A data file has been prepared in which the deposited γ doses for the nuclides making the most important contributions to doses for typical reactor accidents (Ru-103, Ru-106, I-131, Te-132, Cs-134, Cs-137 and Ba-140) were calculated using EXPURT. Doses from other nuclides were calculated using a simpler model which assumes that the dose in the area where people live can be represented by that over an open field. This file has been passed to KfK for inclusion in version 93/1 of COSYMA.

A report describing this work is being prepared. The report also gives advice on the location factor to be used with the data file provided.

1.2 Revised food chain data libraries

Revised food chain data libraries have been prepared using the NRPB model FARMLAND. These datafiles take into account changes to model assumptions and parameter values following a comprehensive review of the FARMLAND model at NRPB, together with the correction of some errors in the previous data libraries. The new foodchain data libraries in COSYMA are therefore consistent with NRPB's current position on foodchain modelling. The data libraries are intended for general application in central and northern Europe, and use agricultural practice assumptions and parameter values agreed by NRPB and GSF as part of a separate CEC contract on derived intervention levels. The files have been passed to KfK for inclusion in version 93/1 of COSYMA.

1.3 Guidance Notes on Meteorological Sampling and Choice of Atmospheric Dispersion Model

Methods of grouping atmospheric conditions for meteorological sampling have been considered in the past in some detail for use with dispersion models which do not consider changes of wind direction during the travel of the plume. However, little guidance is available for use with those models which do consider this (termed 'trajectory models'). To investigate this, four meteorological sampling schemes for use with trajectory models were derived, and several sets of starting times derived from each of the schemes. Runs of COSYMA for each set of starting times were then carried out. The spreads in the predicted consequences for early death and people evacuated for a hypothetical release were considered, and recommendations were then made as to the most appropriate sampling scheme for use with

the trajectory models in COSYMA. This was published[1] as an extra section of the COSYMA user guide.

COSYMA contains five different atmospheric dispersion models, each with different strengths and weaknesses, and different areas for which they are most appropriate. Some of the models require much more computer time and disc storage than others. A number of comparisons of accident consequences predicted using different dispersion models have been carried out at NRPB, KfK and elsewhere. These studies were reviewed, and recommendations given on which model should be used in particular situations when probability distributions of consequences are being calculated. These recommendations were published[1] as an extra section of the COSYMA user guide.

2 PC COSYMA

2.1 Development of Version 1.0

A version of COSYMA for running on an advanced PC has been developed by NRPB and KfK. The system is based on the mainframe version of COSYMA but includes an extensive user interface which guides the user through the process of setting the parameter values required and presenting the results. The user interface has been developed at NRPB, the calculation programs have been developed at KfK. Several versions of both the calculation programs and the interfaces have been exchanged between KfK and NRPB during the development period, and many discussions on the system have taken place during the development. Two documents, a description of the models included in the system[2] and a user guide[3], have been prepared.

Extensive testing of the interface has been carried out at both establishments. Comparisons of the consequences predicted by the PC and mainframe versions of COSYMA have also been carried out at NRPB and KfK. An early version of the system was demonstrated at a CEC conference[4].

NRPB distributes the system as a package consisting of the two reports and 10 disks to other organisations on behalf of CEC. At present 23 versions of the system have been provided to organisations in 15 countries.

2.2 Training Course

NRPB organised a training course in the use of PC COSYMA, with support from CEC. The residential course was held at a hotel near NRPB. It was attended by 37 students from 20 countries, including 11 EC Member States. The course consisted of lectures on the models included in PC COSYMA and the applications for which the code was appropriate, demonstrations on setting up parameter values and presenting results, and sessions where the students used the system. The lectures and demonstrations were given by NRPB and KfK staff.

3 CEC/NEA ACA Code Comparison Exercise

The specification for this exercise was finalised in 1992. Following that, NRPB and KfK agreed suitable input values, and KfK carried out the required runs using version 92/1 of COSYMA, as described in their section of the progress report. NRPB, KfK and UPM staff attended a meeting of a group to discuss and analyze the results produced by the six codes and write a report for submission to the NEA groups CSNI and CRPPH.

Further runs of COSYMA were carried out by NRPB and KfK in connection with a subsidiary intercomparison exercise of COSYMA users, coordinated by KEMA. This aspect is described in more detail in the KfK and KEMA sections of this progress report.

4 Publications

1 J A Jones. Guidance on meteorological sampling in COSYMA *and* The choice of atmospheric dispersion model for use in COSYMA. Added to the COSYMA User Guide, EUR 13045.

2 J A Jones, P A Mansfield, S M Haywood, A F Nisbet, I Hasemann, C Steinhauer, J Ehrhardt. PC COSYMA: an accident consequence assessment package for use on a PC. EUR-14916 (1993).

3 NRPB and KfK. PC COSYMA Version 1.0 - User Guide. EUR-14917 (1993).

4 P Mansfield, J A Jones, S M Haywood, I Hasemann, C Steinhauer and J Ehrhardt. PC COSYMA: A PC version of the probabilistic accident consequence code COSYMA. Poster presentation at the Third International Workshop on Real-time Computing of the Environmental Consequences of an Accidental Release to Atmosphere from a Nuclear Installation, Schloss Elmau, Bavaria, Oct 1992.

Head of project 2: Dr. Ehrhardt

II. Objectives for the reporting period

Within the last 2 to 3 years, the program package COSYMA has proven to be an internationally accepted tool for assessing the radiological and economic consequences of accidental releases of radioactive material to the atmosphere. The OECD-NEA/CEC Intercomparison Exercise on probabilistic accident consequence assessment codes built an important forum for demonstrating its flexibility and reliability. Its widespread use will be intensified by the preparation of the first release 1.0 of its PC version. Maintenance and further development of both the mainframe and the PC version, and support of and interaction with the users, are indispensable for a continuously assured quality of models and data sets incorporated in COSYMA.

III. Objectives for the next reporting period

Work at KfK will concentrate on the maintenance and the further development of both the mainframe and PC version of COSYMA, and the support of and interaction with the users. In particular, the economics module will be completed by a site-specific option incorporated in version 93/2, which will allow to calculate evacuation and relocation costs using information about the economic structure in the vicinity of the nuclear facility. In support of NRPB, models, data and user interface of PC COSYMA will be improved based on the experience gained after the distribution of version 1.0, aiming at a new release 2.0 in 1994.

IV. Progress achieved including publications

1. Maintenance, further development and distribution of the mainframe version of COSYMA

The program package COSYMA jointly developed by KfK and NRPB was released in February 1991 and February 1992 in its versions 90/1 and 91/1, respectively. Some 30 institutes in the EC and other countries got copies of the program package for implementation on their computers together with the updated documentation.

Within the reporting period, some refinements of models and new data sets have been incorporated in COSYMA; together with the correction of errors and modelling weak points identified by the users and the code developers, they built the basis for the release of the new version 93/1, which will be distributed together with the updated user guide to interested institutions in August 1993. The most significant modifications are described below:

1.1 Health effects model

The 'Health Effects Model'/1/ developed in the US builds the basis for the modelling of deterministic health effects in COSYMA as in other accident consequence codes /2,3/. The COSYMA versions released until now, however, neglect the dependence on the dose-rate of the D50-value (i.e. the dose at which half of the irradiated population experiences the health effect considered). For most of the early health effects the radiation doses accumulated at low dose-rates over longer time periods are less effective than those resulting from short term exposure at high dose-rates; therefore, the individual risks have been overestimated by this simplification.

In consultation with NRPB, those dose-rate dependencies have been considered in the 93/1 version of COSYMA, which are quantified in the 'Health Effects Model', i.e. for the hematopoietic syndrome, the pulmonary syndrome and for lung function impairment. For each of these effects, a certain number of i time periods are distinguished, within which the dose D(i) accumulates; the value D50(i) is assigned to each of these time intervals. It is calculated by the formula

$$D50(i) = D0/DR(i) + D_\infty$$

with

$DR(i) = D(i)/T(i)$ average dose-rate in the time interval T(i),

and

$D0$, D_∞ model parameters.

The following default values of D0 and D_∞ have been taken from /1/:

	D0 [Gy²/h]	D_∞ [Gy]
hematopoietic syndrome	0.072	3
pulmonary syndrome	30	10
lung function impariment	15	5

These values can be changed by the user.

The health effect models for all other deterministic effects remained unchanged, i.e. they do not contain any dose-rate dependence.

1.2 Economic model

In order to take into account site-specific economic pecularities for the calculation of loss-of-income costs in case of evacuation or relocation, a special calculation procedure has been developed which is based on the number of employees in different economic sectors of the evacuation/relocation area. The unit costs in this case are specific values for economic production per employee in each economic section.

To perform this kind of cost calculations, an extensive data base has been evaluated for the Federal Republic of Germany (excluding the area of the former German Democratic Republic). This data base provides information on the number of employees in 18 economic sectors for each of the about 8500 municipalities in the FRG. A computer program has been established that allows the transfer of the FRG data into a 500 m x 500 m grid up to a distance of 25 km for the near range of nuclear sites within the FRG. For the far range a data set is available with the respective information on the basis of a 25 km^2 grid according to the "European Grid" that is used in COSYMA.

The new calculation procedure has been incorporated in the NE and NL versions of the ECONOMICS module and will be made available in the COSYMA 93/2 version which is envisaged for distribution on request by late 1993.

1.3 Data sets of dose conversion factors

External doses from groundshine are calculated by multiplying the activity concentration on ground surface by a dose conversion factor giving the dose per unit material deposited. A revised data set containing these values at a series of times for each of a large number of nuclides, including the contribution from daughter products formed after deposition, is provided for COSYMA version 93/1. The new dose conversion factors are appropriate for people staying outdoors and were derived by NRPB as described in the NRPB section of this progress report.

With the revised data for the dose conversion factors, the data sets of activity-risk-coefficients for groundshine have been recalculated and replaced in the COSYMA data base.

1.4 Data sets of food chain data

The activity-in-food default data files version IG-91/AX were replaced by the updated version IG-93/AX, in which apparent errors present in the old data were removed and some assumptions about the agricultural practices were revised. As before, the data were calculated with the dynamic foodchain transport model FARM-

LAND and provided by NRPB. With the IG-93/AX data, new data sets of activity-risk-coefficients for the ingestion pathway were generated.

1.5 Increase of flexibility and user-friendliness

Some months after the distribution of version 91/1 of the program package COSYMA a questionnaire was drawn up by KfK and sent out to all users asking for problems in COSYMA applications and proposals for future improvements. The evaluation of the answers is documented in the report on the COSYMA Users Intercomparison Exercise /4/. The comments given by the users on how to increase the flexibility and user-friendliness are considered in version 93/1 as far as reasonable and manageable. Some examples are: distribution of stable iodine tablets based on dose criterion; possibility to calculate within the same run deterministic health effects and individual doses integrated over a specified short time period; options to specify a cut-off distance for assessing early consequences and to apply an average shielding factor for determining early consequences without considering countermeasures; simplification of the user-input for early countermeasure timing and calculation of individual doses integrated over a specified time period; printout of information on the intervention doses for emergency actions; option to include a printout of the input file in the control output; improvement of printout from the evaluation programs; correction of errors and modification of default values.

2. Preparation of the first version 1.0 of PC COSYMA

A version of COSYMA which is capable of running on an advanced PC has been developed by NRPB and KfK. On the basis of preliminary program versions containing the models taken from mainframe COSYMA and adapted at KfK the mainframe user interface, and the PC-program logic developed at NRPB, discussions took place between both institutes on the improvement of the menu driven user interface and the presentation of the results on the screen. Advice was given by KfK how to structure the input sections and how to combine the various modules according to the user's selections of endpoints. Many test runs were performed at KfK to compare the results predicted by PC COSYMA with those obtained with the mainframe COSYMA version 93/1.

Two documents are available describing PC COSYMA, both published by CEC. The PC COSYMA Main Report /5/ gives an overview of the models contained in the system. The User Guide /6/ explains all menus of the input and results interface and gives further details of the parameters for which values can be specified by the user.

Finally, the first version 1.0 of PC COSYMA comprising the two reports and 10 disks with the software was completed and edited by NRPB. It is available now

through CEC and already distributed to more than 20 institutes. In June 1993 a first training course on PC COSYMA given by staff members of KfK and NRPB was held in the UK.

3. Participation at the OECD-NEA/CEC Intercomparison Exercise on probabilistic accident consequence assessment codes

After the pilot study and several revisions of the task specifications in the preceding period, all Benchmark calculations for tasks C1 to C10 have been performed by KfK/NRPB until August 1992 using COSYMA version 92/1. During an expert group meeting in Brussels in November 1992 the results predicted by the six participating codes were discussed and analysed and a document interpreting the differences was prepared. This draft report was presented at the 4th ad-hoc group meeting in Paris in January 1993 and intensively commented. Based on these discussions, SRD provided the Overview /2/ and the Technical Report /3/ in a draft version which was subject of the final meeting in Madrid in April 1993. In the meantime the final draft version has been sent out to the members of CRPPH and CSNI for comments.

In parallel, the calculations for the COSYMA users intercomparison exercise were performed, in which ten organizations participated and which was coordinated by KEMA. To reach the objectives of this exercise it was not necessary to repeat the tasks already calculated in an earlier phase of the Intercomparison Exercise. Thus, slight differences in the specifications and the use of the COSYMA version 91/1 led to a second set of results delivered by KfK/NRPB for this special exercise. Instead of treating all tasks used in the Code Intercomparison Exercise, the users decided to carry out two extra cases to study specifically the implementation of countermeasure strategies and the effect of different meteorological sampling schemes. A documentation giving a very detailed analysis of the differences in the results was drafted by KEMA and KfK. After discussions with the COSYMA participants about this report in Madrid, the final version is now being published by CEC /4/. Additionally, as a result of this exercise, an international COSYMA users group has been established with its first meeting being held in Arnhem (NL) on 25-26 April 1994.

Publications:

/1/ J.S. Evans
Health Effects Models for Nuclear Power Plant Accident Consequence Analysis
NUREG/CR-4214, SAND85-7185, Rev.1 (1990)

/2/ International Comparison Exercise on Probabilistic Accident Assessment Codes - Overview Report
OECD and CEC, Final Draft, May 1993

/3/ International Comparison Exercise on Probabilistic Accident Assessment Codes - Technical Report
CEC, Report EUR-15109, to be published

/4/ E. van Wonderen, J. van der Steen, I. Hasemann
COSYMA: Users Intercomparison Exercise
CEC, Report EUR-15108 (1993)

/5/ J.A. Jones, P.A. Mansfield, S.M. Haywood, A.F. Nisbet, I. Hasemann, C. Steinhauer, J. Ehrhardt
PC COSYMA: An Accident Consequence Assessment Package For Use on a PC
CEC, Report EUR-14916 (1993)

/6/ NRPB and KfK
PC COSYMA Version 1.0 - User Guide
CEC, Report EUR-14917 (1993).

Head of project 3: Prof. Alonso

II. Objectives for the reporting period (September '92 - August '93)

- **To undertake further work on the development of techniques for assessing the secondary, or indirect, economic impact of nuclear accidents.** Revision and evaluation of different possible methods, and design of the appropriate methodology for use in ACA studies.

- **To incorporate the MECA2 model (Model for Economic Consequence Assessment) into the COSYMA system** as an additional economics module. To conclude the participation with MECA2 in the CEC/NEA ACA code intercomparison exercise. To study the programming of the economics modules in COSYMA.

Objectives for the next reporting period. (September '93 - May '94)

- Application of the selected methodology for the analysis of the indirect economic impact to deterministic calculations using data from specific nuclear sites. Development of a computer program to test the feasibility of the incorporation of that methodology to usual probabilistic ACA codes like COSYMA.

- Incorporation of MECA2 subroutines into COSYMA, testing against the results of the NEA/CEC ACA code intercomparison exercise, and documentation for potential users.

III. Progress achieved including publications

The first part of the project is related with the **analysis of the effects of a nuclear accident on the economic variables of the affected and surrounding areas.** The objective of the research is to evaluate the economic impacts of a nuclear accident on:

- the area directly affected, and
- the areas which have economic relations with the area directly affected.

Two different kinds of variables are distinguished:

a) Variables of FUND, which correspond to the productive capital and the natural resources. The evaluation of the damages will be in terms of replacement costs, although the possibility of evaluating them in terms of lost flows of wealth will also be considered.

b) Variables of FLOW, which correspond to the products of the economic system. The impact over these variables will be established through the use of Input-Output tables, and the Leontieff's production functions.

- The following methodology and hypothesis will be used in the handling of the available Input-Output tables.

HYPOTHESIS:

1. As there are not Input-Output tables at regional level, the technical coefficients will be taken as identical to those at the national level. The implication of this assumption is that the technology and efficiency are the same as at national level.

2. An Input-Output table will be built for the affected area and two more for the surrounding areas, so that the proximity factor is included.

3. The GDP of the affected area will be estimated by multiplying the GDP of the region by the indicator "proportion of active population of the area".

4. The structure of the effective demand of each area will be established according to the structure of the demand at national level, which implies the same patterns of consumption and of investment activity.

5. The impacts over the effective demand of the affected area will be a consequence of:

   - The reduction of the income of its inhabitants.
   - The reduction or increase of public spending in the area.
   - The reduction or abandonment of investment plans.
   - The elimination of imports and exports.

6. The impacts over the economies of the non-directly affected areas will be influenced by the following circumstances:

   - If there is spare production capacity.
   - If there is not production capacity, the balance of payments will be affected.

7. Little impact of the accident over the national economy as a whole. The price formation process will not be affected.

METHODOLOGY:

Combining the technical coefficients of the Input-Output table, the following system of equations can be obtained:

$$\begin{aligned} a_{11}\ I_1 + a_{12}\ I_2 + \ldots + a_{1n}\ I_n + D_{f1} &= O_1 \\ \vdots \quad\quad \vdots \quad\quad \vdots \quad\quad \vdots \quad\quad \vdots \quad\quad \vdots \quad\quad \vdots \\ a_{n1}\ I_1 + a_{n2}\ I_2 + \ldots + a_{nn}\ I_n + D_{fn} &= O_n \end{aligned}$$

where:
- a_{1i} is the technical coefficient = X_{1i}/I_i, being
- X_{1i} the production that sector 1 sells to sector i and
- I_i the input to sector i;
- D_{fi} the final demand of sector i, and
- O_i the output of total production of sector i.

Since for the same sector (i=j) the input is equal to the output ($I_i = O_j$), it results

$$\begin{Bmatrix} D_{f1} \\ . \\ . \\ D_{fn} \end{Bmatrix} = \begin{Bmatrix} (1-a_{11}) & -a_{12} & \dots & -a_{1n} \\ . & . & & . \\ . & . & & . \\ -a_{n1} & -a_{n2} & \dots & (1-a_{nn}) \end{Bmatrix} \cdot \begin{Bmatrix} I_1 \\ . \\ . \\ I_n \end{Bmatrix}$$

which can be abbreviated as

$$\{D_{fi}\} = \{A_{ij}\} \cdot \{I_j\}$$

Once known the final demand $\{D_{fi}\}$ the total productions can be obtained as:

$$\{I_j\} = \{A_{ij}\}^{-1} \cdot \{D_{fi}\}$$

Which means that any modifications of the inputs will be a function of the increments of the final demand:

$$\{\Delta I_j\} = \{A_{ij}\}^{-1} \cdot \{\Delta D_{fi}\}$$

Similarly, the impact over the amount of factors employed $\{F_j\}$ can be obtained as:

$$\{\Delta F_j\} = \{B_{ij}\} \cdot \{\Delta I_j\}$$

where:

$$\{B_{ij}\} = \begin{Bmatrix} (1 - \sum_{j=1}^{n} a_{j1}) & \dots & 0 \\ . & . & . \\ . & . & . \\ 0 & \dots & (1 - \sum_{j=1}^{n} a_{jn}) \end{Bmatrix}$$

The second section of the project aims to **the incorporation of the MECA2 model into the COSYMA system** as an additional economics module. During the reporting period, the following activities have been developed:

1. Participation in the CEC/NEA ACA code intercomparison exercise, using the MECA2 linked to the U.S. ACA code MACCS 1.5, and the COSYMA code itself (in collaboration with the Nuclear Safety Council of Spain). Collaboration with KEMA and KfK in the interpretation of the differences resulting between COSYMA users, specially in the economics results. It has contributed to a better knowledge of the differences in modelling and results provided by both MECA2 and the current economics model (COCO-1) in COSYMA. The cases studied in that Benchmark Exercise will be used for testing the new module once incorporated in COSYMA.

2. Study of the coding of the economic sections within the NE and NL subsystems of COSYMA, in order to start the implementation of the corresponding subroutines and socio-economic database of MECA2, keeping the overall structure of COSYMA unchanged.

Head of project 4: Mr. J. van der Steen

II Objectives for the reporting period

i) To coordinate the CEC/NEA COSYMA users intercomparison exercise;

ii) To analyze the results of the calculations of the participating COSYMA users and to explain the differences;

iii) To report the outcome of the analysis in a EUR report.

The objectives for the next reporting period are:

i) To coordinate the activities of the COSYMA Users Group according to its terms of reference;

ii) To identify weaknesses in COSYMA and PC COSYMA, as identified by the users in their applications of the code, and to advise in the maintenance and further development of the COSYMA system.

iii) To assist in a review of the current position of PCA codes and models, with respect to the needs and priorities for future research.

III Progress achieved including publications

1 Coordination of the COSYMA Users Intercomparison Exercise

In the reporting period, the COSYMA Users Intercomparison Exercise has been finalized. In total 10 organizations took part in the exercise. Some of the participants joined their efforts, which resulted in 7 different sets of calculations. As the exercise was performed in the framework of the CEC/NEA PCA Code Intercomparison Exercise, use was made of the same specifications, with some modifications for the specific aims of the users intercomparison. Some extra cases were added to the calculations, which were specifically designed to study some of the specific features of COSYMA in more detail. These specifications were elaborated by NRPB and KfK, and finalized in a meeting of the COSYMA participants in the Intercomparison Exercise. Participants sent their results to KEMA, who coordinated the activities of the COSYMA Users Intercomparison Exercise. A further meeting was organized to discuss and analyze the results of the calculations.

The exercise has provided a valuable forum for discussion on the use of COSYMA. As a result, an international COSYMA users group has been established, with the purpose of reinforcing the feedback between users and code developers.

2 Analysis of the results

The submitted results of the calculations were analyzed in detail, together with KfK. The majority of the results were in very good agreement, and it appeared to be possible to explain almost all the differences, even if they were very small. Some extra test runs were carried out to verify the explanation of the differences. The outcome of the exercise supported the conclusions of the Code Intercomparison Exercise.

The exercise has been a fairly rigorous test of both the users and COSYMA. This has considerably enhanced the quality assurance aspects of the code and its use. It has given feedback to the owner and the developers on weaknesses in the code and in the user guide and has given advice on future improvements.

3 Report of the COSYMA Users Intercomparison Exercise

A draft report with the results of the participants, together with the completed analysis, was written by KEMA and KfK. This draft was discussed with the participants, and agreed at the final meeting of the exercise. The final text has been submitted to the CEC for publication as EUR report nr. 15108 EN.

Some results of the exercise are of a more general importance in the field of PCA. Therefore it was decided to publish these aspects in a separate paper in the scientific literature. This paper will be written jointly by NRPB, KfK and KEMA and submitted for publication this year.

4 Publications

E.L.M.J. van Wonderen, J. van der Steen and I. Hasemann; COSYMA: Users Intercomparison Exercise. EUR report nr. 15108 EN.

Progress Report

Contract: **FI3P-CT930073** **Sector: C24**

Title: Analysis and modelling of the migration of radionuclides deposited in catchment basins of fresh water systems.

1) Monte ENEA
2) Van der Steen N.V. KEMA
3) Boardman UKAEA

I. Summary of Project Global Objectives and Achievements

The aim of the project is the analysis of the most important processes that influence the transport of radionuclides, introduced into the environment following an accidental release of radioactive substances, through catchment areas to develop generic models assessing the behaviour of radionuclides in drainage basins. The developed models, that account for all possible transport processes, like run-off, wash-out, river transport, etc., will be applied and tested under different environmental conditions.
At present stage the following achievements were reached:
a) A review of the "state-of-the-art" of knowledge of the processes driving radionuclide migration in drainage areas and of the techniques by which these processes have been represented in currently available radiological models;
b) The evaluation of experimental "transfer functions" from drainage areas to waterbodies carried out using data of radionuclide contamination collected in some European rivers; the comparison of these functions and the performances of the literature models offers the opportunity of evaluating what processes are accounted by the existing models and what aspects of the migration process have to be investigated in more details.
c) The collection of data sets, including radionuclide deposition over drainage areas, radionuclide concentration in waters, sediments, and suspended matter, drainage area characteristics, etc., relevant for many European aquatic systems; one of these systems is the river Rhine.
d) The study of the importance of lakes as reserves of available radionuclides in complex drainage areas were started.
A first version of a model predicting the migration of radionuclide in drainage areas was developed and applied to various lacustrine European systems and compared with experimental data of water contamination collected over a period of more then 30 years.
Some aspects of the programme have being considered in close co-ordination with the CEC project "Real time on-line decision support systems for off-site emergency management following a nuclear accident" (COSU-CT91-0020) and with the IAEA/CEC project VAMP (VAlidation of Model Predictions).

Head of project 1: Dr. Monte

II. Objectives for the reporting period

Objective for the reporting period - During the period January - August 1993 the following objectives were reached: a) radionuclide contamination contents in various components of some Italian water systems were collected; b) transfer functions from catchment to water bodies were evaluated using experimental data collected from the scientific literature; c) a first version of a model predicting the migration of radionuclides (^{137}Cs and ^{90}Sr) in catchment was developed. The model was integrated in the **MARTE** (Model for Assessing Radionuclide Transport in Aquatic Environment) model (generic model developed to predict the migration of radionuclides in water systems) and applied to some Italian lacustrine basins (Bracciano, Vico, Garda, etc.). The comparison between the model outputs and ^{137}Cs and ^{90}Sr experimental concentrations collected over a period of more then 30 years (since the end of 50's to August 1993) offered the opportunity of calibrating the model and of evaluating the importance of the different phenomena involved in the migration of radionuclide in catchment.
Objective for the September 93/May 94 reporting period - Phase A: a detailed analysis of the transfer functions of literature models, developed to assess the migration of radionuclides from catchment basins to water bodies, will be carried out to identify the main processes considered by the models used up to now and to identify what aspects of such models have to be improved. Phase B: the comparison between the MARTE model outputs and experimental data will be carried out for other lacustrine systems (Viverone, Bolsena, etc.). The goal of phase A and B will be to acquire information to improve the performances of MARTE model. The importance of the interaction of radionuclide with sediments will be investigated comparing the behaviour of ^{137}Cs and ^{90}Sr (radionuclides characterised by very different values of the water/sediment partition coefficient). The role of lakes in the radionuclide migration through complex catchment will be analysed; indeed lakes represent reserves of available radionuclide in drainage areas.

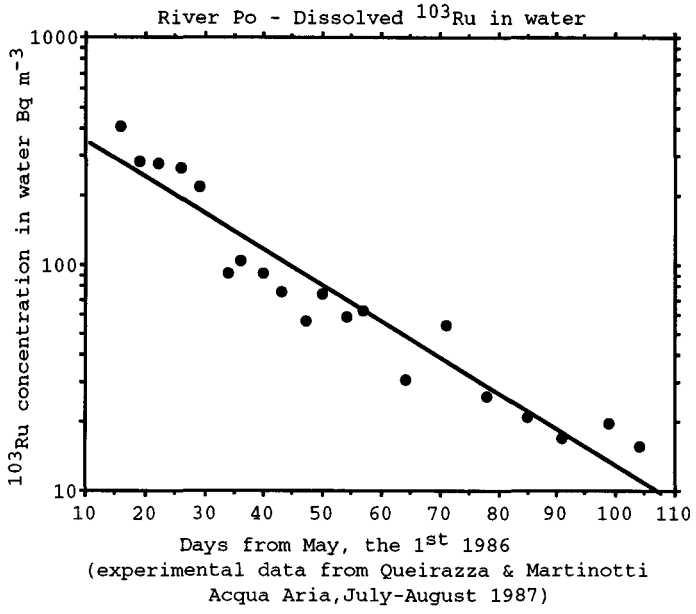
III. Progress achieved including publications

During the period January-August 1993 radionuclide contamination in some Italian aquatic systems was measured. Moreover contamination data collected during the past decades in various aquatic systems and the average ^{137}Cs and ^{90}Sr depositions on Italy due to the fall-out of atomic weapon test and to the Chernobyl accident, were gathered. This phase of the work allows to collect data set of radionuclide contamination to be used in model calibration and validation. At present more then 20 Italian water systems, lakes and rivers covering a wide range of characteristics, have been analysed. The main studied systems are the following: river Sarca(tributary) - lake Garda - river Mincio (emissary), river Adda (tributary) - lake Como - river Adda(emissary) (both systems are located in the North Italy), volcanic lakes of Central Italy (Bolsena and the emissary river Marta; Bracciano; Vico and the emissary stream Vicano; Albano; Nemi), lake

Viverone (North Italy), and a variety of other lakes located in Central Italy (Martignano,Monterosi, Turano, Piediluco etc.). Moreover measurements of ^{90}Sr concentrations in water of river Tevere (Central Italy) were carried out (data of contamination for this river are available since the 1965).
An analysis of data reported by the scientific literature of radionuclide contamination in some European rivers (Po, Prypiat, Dnieper, Teterev, Uzh, Desna, Rhine) following the Chernobyl accident was carried out to evaluate the "transfer functions" of radioactive substance from catchment basins . Two main exponential effective decay components of the radionuclide flux from catchment were detected.
The order of magnitude of the effective decay constants (short term) for ^{137}Cs, ^{90}Sr and ^{103}Ru calculated is 10^{-7} s^{-1}. The geometric means of the effective decay constants for the long term component are 1.2 10^{-8} s^{-1} (^{137}Cs) and 5. 10^{-9} s^{-1} (^{90}Sr). A significant non linearity of ^{90}Sr transfer functions respect to the water flow through the drainage basin was detected.
As example, the enclosed figure shows the time behaviour of ^{103}Ru in river Po; the table reports the values of the long term effective decay constant evaluated.
A preliminary literature analysis of "radionuclide transfer functions" from catchment, used up to now by models developed to assess the migration of radioactive substances in catchment basins, showed that the models reported by the scientific literature not ever accounts for these components for possible non-linearity effects.
The experience gained by the analysis of the experimental transfer function allowed to develop a first simple model of the migration of radionuclides in catchment .
This model is based on the existence of two main compartments: a first "fast" compartment accounting for the effect of radionuclide wash-out from vegetation or, more generally, for the effect related to the very mobile component of radionuclide following its deposition above ground; a second "slow" compartment accounting for the rain-out removal of radionuclide (or, more generally, effect related to the less mobile component of radionuclide).
The above model was integrated in a model for predicting the behaviour of radionuclides in aquatic systems (**M**odel for **A**ssessing the **R**adionuclide **T**ransport in Aquatic **E**nvironment: **MARTE**). MARTE model account for the following processes: direct deposition of radionuclide onto water surface; adsorption of radionuclide by suspended matter; radioactive decay; transport of radionuclide in dissolved and in particulate form by water flow; sedimentation, diffusion of radionuclide in sediments.
The model, at present stage, includes a variety of phenomena ranging from the catchment migration to the behaviour of radionuclide in water body. The model was successfully applied to some Italian lacustrine systems (lake Bracciano, Vico and Garda).

River	Radionuclide	α (s^{-1})	$\lambda_2+\lambda_r$	r^2	Period of collection of fitted data (day after May 1st, 1986)
Desna	^{137}Cs	1.0	$6.2\ 10^{-9}$	0.693	240 - 1700
Dnieper	^{137}Cs	0.86	$1.1\ 10^{-8}$	0.712	240 - 2000
Prypiat	^{137}Cs	1.08	$1.8\ 10^{-8}$	0.819	240 - 2000
Rhine	^{137}Cs	0.53	$2.7\ 10^{-8}$	0.861	240 - 600
Teterev	^{137}Cs	0.96	$8.2\ 10^{-9}$	0.592	240 - 1700
Uzh	^{137}Cs	1.02	$1.5\ 10^{-8}$	0.762	240 - 1700
Geometric	mean	0.89	$1.2\ 10^{-8}$		
Desna	^{90}Sr	1.1	$5.4\ 10^{-9}$	0.664	240 - 1700
Dnieper	^{90}Sr	1.4	$5.5\ 10^{-9}$	0.656	240 - 2000
Prypiat	^{90}Sr	1.41	$4.9\ 10^{-9}$	0.679	240 - 2000
Teterev	^{90}Sr	1.12	$3.6\ 10^{-9}$	0.586	240 - 1700
Uzh	^{90}Sr	1.31	$5.9\ 10^{-9}$	0.827	240 - 1700
Geometric	mean	1.26	$5.\ 10^{-9}$		

Evaluation of parameter values of the slow component of the transfer function for some European rivers. α = exponent of water flux; $\lambda_2 + \lambda_r$ = effective decay constant of the slow component; r = correlation coefficient between the natural logarithm of radionuclide flux (Bq s^{-1}), the natural logarithm of water flow ($m^3\ s^{-1}$) and the time. The analytical form of the slow term of the transfer function here used is the following: $\Phi_r(t) = K\ \Phi(t)^{\alpha} e^{-(\lambda_2 + \lambda_r)t}$; K is a constant depending on the deposited radionuclide, $\Phi(t)$ is the water flow.



Publications

Model MARTE is partially described in

Luigi Monte - A predictive model for the behaviour of radionuclides in lake systems. Health Physics 65(3):288-294.

Data relevant to the transfer function evaluation are repored in

Luigi Monte - Evaluation of radionuclide transfer functions from drainage basins of fresh water systems. To be published

Head of project 2: Mr. J. van der Steen

II. Objectives for the reporting period

Progress over the reporting period

Data collection

The first step in the project is the evaluation of the pulse after the accident to describe the radionuclide transport in the catchments with a simple transfer function in which the catchment area is considered as a black box. This transfer function can be correlated with characterisation of the catchment, including e.g. the land use and soil properties. All possible hydrological, geochemical and radiological data have to be collected for that purpose.

Hydrological data of the Rhine catchment are collected by means of contacts with institutes in Germany and Switzerland. This includes information about the catchment concerning water discharges and suspended sediment concentrations in the River Rhine and its tributaries. Soil maps with extended descriptions of the properties of the different soil types in the Rhine drainage area supplies information about the possible migration of nuclides from soil to land. Geochemical maps add information on the presence of other pollutants and elements, which may influence the uptake and downward transport of radionuclides in the catchment system. Collection of data concerning the radionuclide concentration in soil after the Chernobyl accident to calculate the transfer from catchment to the rivers, and the collection of data concerning the river and lake system takes place during the reporting and next project period. This includes radioactivity levels in suspended sediment, water, bottom sediments, and different soil layers, as function of space and time.

Measurement on geochemical aspects of the catchment area.

Measurement of stable caesium in sediment had been carried out to investigate the role of stable caesium in the adsorption behaviour of radionuclide in the river. Discharges of salt into the Rhine might diminish the uptake by sediments, which could results into a lower residence time of radioactive cesium in the aquatic system after an accidental release.

Objectives for the next period.

Subdivision of the catchment into homogeneous areas based on the collected data is a topic of the next project period. From the literature study within the project on runoff models a model type will be selected and adapted on the Rhine system to predict the short-term runoff. Afterwards a model, probably a box-type model, will be developed for the long-term land-to-water transfer. Preliminary studies on the effect of the release of radionuclides by resuspension, erosion, and weathering will be started. The influence of the chemical compounds on sorption processes in soils and sediments will be studied in order to identify the most important parameters which influence the transport through the catchment.

Interaction with other projects.

Information and conclusions from the hydrological subgroup of JSP1, a part of the CEC-project "Real-time on-line decision support systems for off-site emergency management following a nuclear accident" (COSU-CT91-OO20), will be used for constructing the aquatic model for Rhine catchment area.
Conclusions and results from studies within the frame of this project concerning geochemical properties of the catchment that are important for the several models developed within this project can be used for adjusting the processes modelled within the frame of JSP1, especially to adjust the land-to-water transport.
Results and data from VAMP (VAlidation of Model Predictions) project on the behaviour of radionuclides on the different lake systems in Europe can also be used to get more insight in the processes involved in the transport from catchments to lakes. Vice versa results from studies on the effect of geochemical condition can be used in the VAMP project.

Head of project 3: Miss J. Boardman

II. Objectives for the reporting period

Non-gaseous radionuclides released to the atmosphere will ultimately be deposited on land or water surfaces by such natural processes as settling and scavenging by rainfall. Although a fraction of these radionuclides will fall directly onto water surfaces, a proportion of those that are deposited on land could be carried to surface waters by runoff or via infiltration and subsequent groundwater transport. The objectives for the current reporting period were two:

(i) gather information on the likely processes driving radionuclide migration from drainage/catchment areas, and

(ii) to begin to review the techniques by which these processes have been represented in currently available radiological models.

The above activities have taken account of information provided by each of the participants in the collaboration.

During the next reporting period, the UKAEA model of the freshwater environment will be improved based on the conclusions of the catchment review.

III. Progress achieved including publications

A review of the literature describing the processes involved in radionuclide migration from the freshwater catchment to surface water bodies has been started. The resulting simulations of the radionuclide behaviour within currently available mathematical models have also been considered. Some preliminary conclusions are presented below.

A. Important Processes

A catchment is defined as the area of land bounded by watersheds draining into a river or lake. Water enters the catchment primarily as precipitation (including snowfall), though artesian upwelling of groundwater may also be of significance. Losses are via evapotranspiration, surface runoff and subsurface drainage to a surface water body, and percolation to the deep groundwater system.

The near-surface hydrological system is both spatially and temporally heterogeneous on a wide variety of scales. Hydrological properties of the catchment are strongly conditioned by topology, vegetation cover, soil characteristics, underlying lithology and drainage patterns. Surface slopes govern runoff processes, while plants act both to intercept precipitation before it reaches the soil and to extract water from the soil zone for that lost by transpiration.

Within soils it is often convenient to distinguish an upper undersaturated zone from a lower saturated zone, the boundary between these being the water table, or phreatic surface. Infiltrating water movement in the undersaturated zone is predominantly vertical. Conversely in the saturated zone the flow is predominantly horizontal, with the underlying lithology influencing the degree of vertical movement possible. In areas of complex lithology complications arise with, for example, the presence of perched water tables, while the soil structure has a major effect on hydrological properties, e.g. through the presence of macropores.

The transport of radionuclides following deposition on to the catchment, through to a surface water body is associated with the movements of water and particulate above and beneath the soil surface, while transfer of activity between the two phases occurs by sorption from solution onto soil particle surfaces. This transfer between solid and liquid phases is most often described very simply by the distribution coefficient, K_d, which relates the concentration of adsorbed activity to that in solution. However the efficiency of sorption processes exhibited in the environment, which link transport in the particulate and liquid phases, is highly variable and complex. The process generally depends on factors such as solubility of the element, available surface area (depending on particle size), mineralogy (e.g. competing cations) and the organic content of soil. Given the range of parameter and conceptual uncertainties inevitably associated with modelling catchment water and sediment yields, for many radionuclides it is uncertainty and spatial variation in K_d that most limits the accuracy with which the loss of deposited radionuclides from a catchment to an adjacent water body can estimated.

One thing we hope to determinate with our future modelling is the relative importance of groundwater (sub-surface) flow and overland flow of water (which may also drag and transport surface soil) in transporting radionuclides towards the receiving watercourse. Although water velocity of overland flow is much greater than groundwater, a greater volume of water travels below the surface to the receiving watercourse. Thus for labile nuclides groundwater transport could be significant, especially over long term. Another question we hope to answer is the relative importance of overland flow of water to that of sediment for UK catchments.

B. Modelling Approaches

Empirical Models/Exponential Fitting

A number of researches have measured various catchment responses to atmospheric fallout data and explain their observations in terms of a fast and slow component of transport (e.g.[1]). "Fast" response of the catchment is generally attributed to overland erosion of recently deposited fallout due to overland flow of rainwater - a process known as direct runoff. "Slow" response is attributed to leaching of activity within the soil by rainwater and subsequent subterranean transport toward the receiving water course. Compartment or box models are thus developed assuming that the measured response of the

particular catchment to an initial deposit of activity can be represented by the sum of two (or more) exponential (e.g. [2], [3], [4], [5]). The main application of these models is that the "best fit" (to data) response curve can be extrapolated in time to predict long term behaviour of the catchment. Joshi [3] has also extended the technique so that the response to other radionuclides for which there is no data can be inferred in terms of the radionuclide distribution coefficient K_d.
However, little attempt has been made to explain the results in term of measurable catchment characteristics such as soil type, slope or vegetation, and as result these models have limited application to other drainage areas for which no monitoring data is available. For our work we have concentrated on developing models which represent catchment response in terms of a small set of parameters characterising the drainage area.

Conceptual and Quasi-Physical Models

The development of a more physically based model to assess the yield of initially deposited radionuclides to an adjacent water course generally follows three stages:

i) The development of a surface hydrology model, describing flows above and below the soil surface, losses to plants and the atmosphere as well as to deep ground water.

ii) Development of a sediment yield model. This can be developed as an overlay to the overland flow component of the hydrological model, in which soil grains are detached from the soil matrix by raindrop impact and are transported downslope during periods of heavy downpour.

iii) A model to describe the transfer of radionuclides between mobile water flows and generally less mobile particulate.

Such models (e.g. [6], [7]) evaluate the catchment response predictively in terms of actual, measurable, catchment characteristics.

Hydrology models that have previously been applied to radiological assessments fall into two categories. Firstly there are such models as the Stanford Watershed Model. These models are often referred to as conceptual models and are based on the Explicit Soil Moisture Accounting (ESMA) technique, which represents the hydrological cycle as a system of stores between which water flows [8]. Secondly, with advances in computer technology a new class of computationally and data intensive models such as Système Hydrologique Européen model (SHE) [9] have become increasingly popular. These models represent heterogeneous hydrological processes in the catchment using theoretically defensible equations. These are discretised and solved numerically over a finite element mesh which physically representing the catchment.

In developing a generic model to simulate the response of the catchment for deposited activity, the adoption of either approach has its drawbacks.

In using the conceptual approach, the fact that parameters used to support the model are often empirical, and sometime lack a physical basis, can make data elicitation problematic. By using semi-empirical methods there is obviously considerable scope for expert judgement [10]. This is less of a problem for the more physically based models, whose variables have physical meaning, and therefore can be measured directly in the field or expressed in terms of basic physical considerations. A second problem with conceptual models is that they often fail to adequately describe the spatially distributed characteristics of the catchment. This is most problematical when information on the dynamics of activity discharge from the catchment is considered to be important.

However physically-based models also have their disadvantages. The first is that they are often user and computer time intensive, making sensitivity studies impractical to conduct. In addition the lack of resolution due to parameter uncertainty, especially in K_d s, often exceeds the resolution claimed by the model. Thirdly, the fact that they are physically based does not necessarily imply accuracy, since little research has yet been conducted to validate models used in the real environment.

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Progress Report

Contract: **FI3P-CT930077** **Sector: C24**

Title: Multifractal Analysis and Simulation of Chernobyl Radioactive Fallout in Europe

1) Ratti University of Pavia (Italy)

2) Schertzer CNRM (France)

I. Summary of Project Global Objectives and Achievements

The present Contract FI3P-CT930077 concerns an *a priori* analysis (quality assurance) of the available European radioactivity data, since the selection of reliable measurements and networks is needed in order to provide certified and consistent samples as a starting point for any further modelling. This operation requires the estimate of the multifractal features of the radioactive fall-out along with the calculation of the influences and the effects of the corresponding networks; clearly, this step will allow us to identify the processes generating the fall-out in terms of the relevant multifractal parameters. At the same time, given the *turbulent* (and hence *multifractal*) nature of all the geophysical fields playing a significant role in dispersing the pollution, *new statistical techniques* will be developed to *estimate* and/or *forecast* the intensity of the investigated phenomena. It has recently been realized that the standard recovering methods fail to provide meaningful answers when dealing with multifractal fields measured on (multi)fractal networks (as it is the case of post-Chernobyl fall-out over Europe).

The above tasks have to face several formidable difficulties, both practical and theoretical. On the one hand, the data bases are often *statistically poor*, since only a limited amount of data is available to estimate the relevant parameters of interest here and the data themselves need to be checked with accuracy to avoid misuse of information. On the other hand, given the very particular nature of the probability distributions involved, no standard mathematics can be exploited and the theoretical apparatus must be rebuilt from anew. Nevertheless, preliminary results on simulated fields and networks show that the proposed multifractal approach is suitable to overcome many of the listed problems.

A further step foreseen is the creation of a *global framework* describing the relationships between the measured pollution and any relevant meteorological data (e.g. rain, wind, pressure, ...). Clearly, this task is of primary importance in the area of *recovering* and/or *forecasting* . At the same time, it would allow us to exploit the maximum amount of information provided by already existing sources (e.g. National Meteorological Institutes) and hence these new techniques might greatly improve the present predicting schemes.

Head of Project 1: Prof. Ratti

1.II. Objectives of the reporting period

The University of Pavia is taking care of the *a priori* analysis of the available radioactivity data, performing *quality assurance* operations and selecting reliable measurements and/or networks. Thus, the estimate of the multifractal features of the radioactive fall-out will be obtained, along with the calculation of the influences and the effects introduced by the presence of the measuring networks. Furthermore, an empirical multifractal model able to estimate the ^{137}Cs soil distribution starting from the actual measurements of pollution will be tested in those European Countries where statistically significant data are available. Finally, the University of Pavia will specifically develop *multifractal interpolating techniques* to obtain both *local recovery* of the pollution and estimates of its *global statistics*.

1.III. Progress achieved including publications

During this Project a monofractal descriptive model of ^{137}Cs in air in Northern Italy (developed within the EEC Contract BI7*-CT90-0062-MNLA) is exploited again in order to achieve two essential goals. The first one concerning a calibration of the evaluation-uncertainty and the second one concerning the quantitative comparison of the numerical output with certified data-set not used in the development of the model. Then, the systematic multifractal analysis of the European ^{137}Cs soil deposition measurements collected in different Countries is carried out. The data are processed according to the procedures presented in the Final Report of the Contract BI7*-CT90-0062-MNLA and a modelization of the ^{137}Cs soil deposition in several European Countries is performed and discussed. Furthermore, the validity of our model is checked first by running the model using, as input data, reduced sets of the original data-base and, then, by comparing the results to the modelization obtained by using the whole data-set. Finally, new statistical techniques for estimating sparse multifractal fields are introduced; we first give a theoretical introduction to the subject, based on the mathematics of universal multifractals, and then we provide an original procedure to fill up (in space and in time) sparse sets of measurements.

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Head of Project 2: Prof. Schertzer

2.II. Objectives of the reporting period

The Centre National de Recherches Scientifiques is taking in charge the problems of spatial/temporal evolution and of prediction, which are rather challenging and difficult to deal with. However, a powerful multifractal framework (based on *Universal Multifractals*) has recently been developed to deal with complex interacting fields. This new techniques will allow to simulate and analyse the *scaling* relations between the different components of vectorial or tensorial fields (which is the general case of meteorological phenomena) on a wide range of scales. These techniques will be properly readjusted in order to dynamically simulate (in space and time) the radioactive fall-out of interest here, given other information about the actual meteorological conditions. Finally, the preparation of efficient and operative system independent computer code is also foreseen.

2.III. Progress achieved including publications

During this Project we first give a theoretical presentation of the framework of *Universal Multifractals*; this is needed in order to clarify and highlight some points of interest of the mathematical apparatus exploited, such as the *multifractal clouds modelling* and the qualitative understanding of the "hot spots" with the help of *multifractal phase transitions.*

Then, since in fact scalar cascade processes need not to be restricted only to positive scalar fields, we develop a rather general framework which opens a *scaling and vectorial* alternative to *General Circulation Models* (G.C.M.) techniques. Thus, we may then consider and use the *generator* of the joint fields (e.g. velocity, rain rate, radiance, ...) which generates not only each component field, but also their (vectorial, tensorial, ...) interrelations. All the above opens the possibility of making some relevant progress, both theoretical and practical.

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IV

KOORDINIERUNGSTÄTIGKEIT

COORDINATION ACTIVITIES

ACTIVITES DE COORDINATION

IV. Coordination

Study Group meetings, Workshops, Seminars and Symposia have proved to be a most effective means of coordination because they are naturally adapted to scientific work and easily accepted by scientists. These meetings, focusing on the evaluation of particular subjects areas of the Radiation Protection Actions are attended by research workers involved in the contract programme, as well as scientists from non-participating laboratories or organizations and by scientific staff members of the Commission.

On the following pages the various meetings held in the period from 1 December 1992 - 31 October 1993 are listed:

A: Meetings of Study Groups, where scientists involved in the contract programme, independent experts and Staff Members of the Commission discuss specific subject areas of the programme.

B: Meetings organized, coorganized or cosponsored by the Commission of the European Communities on special subject areas of interest for radiation protection and where contacts among scientists from a wider range of discipline and countries might be established.

C: Meetings of experts appointed for the purpose of coordinating and stimulating efforts toward practical measures of radiation protection as foreseen in Chapter III of the EURATOM Treaty or convened by the Commission for special tasks.

☞ For full names of acronyms and abbreviatons please see chapter VII.

Meetings of Study Groups

Study Group on thyroid cancer in children living near Chernobyl

Brussels (B), 21 - 22 December 1992
Panel Members from 12 countries and the Commission

Principal subjects:

- Follow-up of the Panel mission to Minsk, Belarus;
- Finalize the Consensus Opinion on childhood thyroid cancer in Belarus;
- Semi-finalize the CEC report on Thyroid Cancer in Children living near Chernobyl: Expert Panel Report on the consequences of the Chernobyl accident;
- Development of experimental collaboration projects on thyroid cancer.

Study Group on Uncertainty Analysis of Accident Consequence Codes (CEC/US NRC Project)

Chilton (UK), 21 - 22 January 1993
13 participants from 3 countries and the Commission

Principal subjects:

- Expert judgement elicitation
- Uncertainty analysis
- Application of methodology to health effects, environmental transfer, urban pathways and metabolic models

Study Group on CEC/NEA Intercomparison of Probabilistic Accident Consequence Assessment Codes: meetings of Project Management (PMG) and ad-hoc Groups (AHG)

Paris (F), 26 - 29 January 1993
39 participants from 15 countries, OECD/NEA and the Commission

Principal subjects:

- Intercomparison of probabilistic accident consequence codes
- COSYMA and MACCS users' groups
- Review of results of intercomparison
- Preparation of overview and technical reports

Study Group on Uncertainty Analysis of Accident Consequence Codes (CEC/US NRC Project)

Albuquerque (USA), 8 - 10 February 1993
12 participants from 3 countries and the Commission

Principal subjects:

- Expert judgement
- Case studies for elicitation of experts in atmospheric dispersion and deposition
- Pre- and post-processors for uncertainty analysis

Study Group on marine radioecology, including problems around the Arctic Sea

Madrid (E), 28 February - 2 March 1993
10 participants from 7 countries and the Commission

Principal subjects:

- Discussion of the progress made by the different participants
- Plans of works of the initial group and of the newly added institutes
- Presentation by the Norwegian and Swedish scientists regarding the situation in the Arctic zone

Study Group on Decision Support Systems for Off-site Emergency Management (JSP1)

Obninsk (Russia), 2 - 4 March 1993
26 participants from 6 countries and the Commission

Principal subjects:

- RODOS decision support system
- Off-site emergency management
- Data bases of early environmental measurements
- Transfer of hardware/software configuration

Study Group of RODOS Management Group

Karlsruhe (D), 17 March 1993
6 participants from 4 countries and the Commission

Principal subjects:

- Establishment of RODOS Project Management Group
- Quality assurance of software products
- Documentation
- RODOS newsletter
- Integration of other projects and PECO contractors

Study Group on the Behaviour in Forests and Semi-Natural Ecosystems

Thessaloniki (GR), 23 - 25 March 1993
10 participants from 6 countries and the Commission

Principal subjects:

- Presentation and evaluation of a joint intercomparison experiment called the "Uppsala project".
- Presentation and discussion of the ongoing research in the different groups.
- Discussion of the replies received upon a questionnaire concerning opinion of the members of the group on the future of the research on the semi-natural environment and its contribution to the dose to man now and on the long-term.
- Discussion about interactions which could stimulated among related but different projects.

Study Group on "What's new and what's needed in radiation effects research"

Bad Honnef (D), 29 March - 1 April 1993
45 contractors from 12 countries, M. Frazier (US DOE) and the Commission

Principal subjects:

- To bring together physicists, radiation chemists, researchers working in molecular and cellular radiobiology and in animal research together, as well as epidemiologists.
- The necessity for multidisciplinary approach in research aimed at understanding the mechanisms of radiation induced changes in animals and humans.
- To identify what is new and what is needed in radiation effects research for the forthcoming framework programme.
- Draft a report of the meeting to be published by the Commission.

Study Group on Data Analysis in Quality Control and Radiation Protection of the Patient in Diagnostic Radiology and Nuclear Medicine

Brussels (B), 18 April 1993
8 participants from 5 countries and the Commission

Principal subjects:

- Preparation of the Workshop on subject area
- Selection of abstracts of proffered papers
- Subject areas and scope of invited papers

Study Group on Optimisation of Radiation Protection of the Patient

Brussels (B), 19 - 21 April 1993
37 participants from 13 countries and the Commission

Principal subjects:

- Presentation of ongoing research work of contracts with the CEC
- Establishment of cooperation plans
- Establishment of subjects for specific study groups
- Role and responsibility of contract coordinator
- 1994-1998 research priorities

Study Group on CEC/NEA Intercomparison of Probabilistic Accident Consequence Assessment Codes: meeting of programme management group (PMG) and ad-hoc group (AHG)

Madrid (E), 27 - 29 April 1993
50 participants from 15 countries, OECD and the Commission

Principal subjects:

- Probabilistic accident consequence codes
- Intercomparison of code predictions for postulated accidental releases from thermal reactors
- Quality assurance of codes
- Users groups for the codes COSYMA and MACCS

Study group on Uncertainty Analysis of Accident Consequence Codes: Elicitation of Experts in the Areas of Atmospheric Dispersion and Deposition (CEC/US NRC Project).

Albuquerque (USA), 24 - 27 May 1993
28 participants from 6 countries and the Commission

Principal subjects:

- Uncertainty analysis of accident consequence codes
- Expert judgement
- Elicitation of experts in atmospheric dispersion
- Elicitation of experts in deposition

Study Group "Programme Committee on the International Symposium on Remediation and Restoration of Radioactive-contaminated Sites in Europe"

Antwerp (B), 22 June 1993
10 participants from 8 countries and the Commission

Principal subjects:

- Inventory of contaminated sites in Europe
- Criteria for remediation and restoration
- Techniques for restoration
- Selection of papers and arrangements for symposium

Study group - 5th meeting of the IAEA/CEC CRP on "Validation of Models for Radionuclide Transfer in the Terrestrial, Urban and Aquatic Environments (VAMP)"

Vienna (A), 5 - 9 July 1993
75 participants from 27 countries, IAEA and the Commission

Principal subjects:

- Model validation and performance
- Environmental impact assessments
- Terrestrial environment models
- Aquatic environment models
- Urban environment models
- Multiple pathways models

Study Group - Preliminary meetings to establish the scope and content of Projects JSP5 (pathways and dose) and JSP6 (cartography).

Moscow (Russia), 30 August - 3 September 1993
26 participants from 5 countries and the Commission

Principal subjects:

- Pathway analysis and dose estimation for contaminated areas around Chernobyl
- Development of reliable models for dose estimation and their validation
- European atlas of radioactive contamination and external dose from the Chernobyl accident
- Atlas of other levels of radiation or contamination from natural or other sources (eg, weapons fallout)

Study Group on Resuspension and Contamination of Surfaces (ECP1)

Kiev, Ukraine, 22 - 24 September 1993
14 participants from 8 countries and the Commission

Principal subjects:

- Wind driven and technogenic resuspension
- Transfer of deposited contamination by resuspension
- Resuspension during grass and forest fires
- Distribution of particle sizes in resuspended material
- Public and occupational health impact of resuspension

Study group of RODOS Management Group

Karlsruhe (D), 28 - 29 September 1993
5 participants from 4 countries and the Commission

Principal subjects:

- Decision support system for off-site emergency management (RODOS)
- Atmospheric dispersion, foodchain, hydrological and countermeasures modelling
- Collaborative programme with the CIS
- Documentation of system and software

Study Group on Evaluation of strategies and intervention levels for mitigation of the consequences of the Chernobyl accident (JSP2)

Paris (F), 21 - 22 October 1993
37 participants from 8 countries and the Commission

Principal subjects:

- Conceptual basis of intervention policy
- Social and psychological impacts of countermeasures
- Decision support system to evaluate countermeasures' strategies
- Dose distributions in settlements contaminated from the Chernobyl accident
- Historical portrayal of countermeasures taken following the Chernobyl accident

Meetings Organized or Coorganized by the CEC,

EURADOS Working Group 11

Organised by CEC
Luxembourg (L), 22 - 23 March 1993
15 participants from 8 countries and the Commission

Principal subject:

- Radiation exposure of civil aircrew.

Seminar: Molecular Mechanisms in Radiation induced Mutagenesis and Carcinogenesis

Co-organised with US DOE Office of Health and Environmental Research and Dutch Institute for Public Health and Environment
Doorwerth (NL), 19 - 22 April 1993
75 international scientists from 15 countries and the Commission

Principal Subjects:

- DNA Repair and Mutation
- Induced Instability,Cytotoxicity, Mutation
- Recombination and Mutagenesis
- Molecular Analysis of Mutations
- High LET Induced Mutations
- Hereditary Mutations
- Mechanisms in Solid Tumours
- Mechanisms in Leukaemia/Lymphoma
- Modelling Oncogenesis

4th European Seminar on Radiation Protection Optimisation

Organised by CEC
Luxembourg (L), 20 - 22 April 1993
187 participants from 29 countries, OECD and the Commission

Principal subjects:

- Optimisation in occupational exposure
- Optimisation in radioactive effluents
- Optimisation in complex situations
- Optimisation in regulation

Workshop on Individual Monitoring of Ionizing Radiation: The Impact of Recent ICRP and ICRU Publications

Co-organised by the CEC, the European Radiation Dosimetry Group (EURADOS), the Paul Scherrer Institut (CH) and the US DOE (US).
Villigen (CH), 5 - 7 May 1993
120 participants from Member States, Switzerland, Sweden, USA, Canada, Japan, Russia, Poland, Czechoslovakia and Hungary

Principal subjects:

- Aspects of ICRP 60 and ICRU 47 of relevance to individual monitoring
- Radiation quantities: their interrelationship
- Significance of angular and energy distributions of the radiation field
- Required accuracy and dose thresholds
- Performance requirements and tests
- Quality control and quality assurance

International Intercomparison of criticality accident dosimetry systems

Co-organised with CEA (F)
Valduc (F), 7 - 18 June 1993
53 participants from 15 countries and the Commission

Principal subjects:

- Calibration of accident dosimetry systems.
- Irradiation of dosemeters in a reactor under simulated criticality accident situation.
- Assessment and evaluation of measurements.

Workshop on Dynamic Processes in Radioecology

Jointly organised with GSF (D)
Bad Honnef (D) 21 - 24 June 1993
39 participants from EC countries, 1 from Rumania and 1 from the Commission

Principal subjects:

- General aspects of radioecology
- Deposition, interception and resuspension
- Soil-plant transfer
- Plant-animal transfer
- Aquatic ecosystems
- Natural and semi-natural ecosystems
- Urban environment
- Countermeasures

Group of experts referred to in Article 31 of the Euratom Treaty

Luxembourg (L), 22 June 1993
25 participants from 11 countries and the Commission

Principal subjects:

- Revision of the basic safety standards.
- Radiation risks and radiation protection of civil aircrew.
- Industrial gammagraphy.
- Manual on radiation protection for transport workers.

Workshop on Indoor Radon Remedial Action. The Scientific Basis and the Practical Implications

Co-organised with the Ente per le Nuove Tecnologie, l'Energia e l'Ambiente; the Istituto Superiore di Sanita; the International Centre for Theoretical Physics; the International Centre for Theoretical and Applied Ecology; the United States Department of Energy and the United States Environmental Protection Agency.

Rimini (I), 27 June - 2 July 1993
212 participants from 32 countries and the Commission

Principal subjects:

- Mitigation Measures - General Aspects
- Mitigation Measures - Practical Approaches
- Health Effects - Biological Studies
- Health Effects - Physical Factors
- Radon Sources - Ingress Studies
- Radon Sources - Mapping and Geology
- Radon Sources - Methods Measurements
- Policy Matters - Surveys and Outcomes
- Policy Matters - Risks and strategies

ECURIE - M.S. Representatives

Organised by CEC
Luxembourg (L), 5 - 7 July 1993
36 participants from 12 countries and the Commission

Principal subjects:

- Preparation of ECURIE Users Guide
- Review of exercises and future programme
- Software

4th L.H. Gray Workshop on Microbeam Probes of Cellular Radiation Response

Co-organised with the L H Gray Trust (UK)
Northwood (UK), 8 - 10 July 1993
40 scientists from 12 countries (Europe, Japan and USA) and the Commission

Principal Topics:

- Rationale for microbeams: protection level studies
- Rationale for microbeams: Mechanistic studies
- Rationale for microbeams: Targeted therapy
- Particle microbeam approaches
- X- ray microprobes
- Collimation, Detection and Imaging
- Biological experiments using microbeam, probes and single cell assays

EURADOS Working Group 11

Organised by the CEC
Prévessin (F), 27 July 1993
5 participants from 4 countries and the Commission

Principal subject:
- Radiation exposure of civil aircrew.

Workshop on Intakes of Radionuclides: Detection, Assessment and Limitation of Occupational Exposure

Jointly organised by the CEC, US DOE, NRPB and IPSN.

Bath (UK), 13 - 17 September 1993
120 scientists from 18 countries and the Commission

Principal subjects:

- To review existing information on the different physico-chemical and biokinetic characteristics of radioactive material encountered at the workplace.
- To develop approaches for a better definition of site-and material-specific Annual Limits of Intake (ALIs), their range of application and the methods available for demonstrating compliance with dose limits.
- The proceedings of the meeting will be published as a special issue of the journal Radiation Protection Dosimetry.

International Symposium on Molecular Mechanisms of Radiation and Chemical Carcinogen Induced Cell Transformation

Co-organised with US DOE Office for Health and Environmental Reserach
Mackinac Island, Michigan (USA), 19 - 23 September 1993
70 scientists working in cell transformation from 15 countries and the Commission

Principal Subjects:

- Human and Animal Cell transformation systems
- Overview of Cell Transformation Systems
- Mechanisms of Radiation-Induced Cell Transformation and Modulation Factors
- Radiation-Induced Cell Transformation
- Role of Gene Expression in Cell Transformation
- Gene Expression of Cell Senescence and Acquisition of an Infinite Lifespan

Workshop on Data analysis in quality control and radiation protection of the patient in Radiodiagnostic Radiology and Nuclear Medicine

Jointly organised by the CEC, the Word Healt Organisation and the Unitá Sanitaria Locale N°7, Udine (I)
Grado (I), 29 September - 1 October 1993
145 participants from 12 EZuropean and non-European countries and the Commission

Principal subjects:

- Evaluation of a decade of research and the existence of the Council Directive concerning radiation protection of the patient.
- Prospectives and priorities in the subject area.

International Symposium on Remediation and Restoration of Radioactive Contaminated Sites in Europe

Co-organised by the CEC, SCK/CEN Mol (B) and the State Committee of the Russian Federation on Chernobyl Affairs
Antwerp (B), 11 - 15 October 1993
196 participants from 27 countries, IAEA and the Commission

Principal subjects:

- Inventory of radioactive contaminated restoration of sites
- Conceptual basis of and criteria for remediation
- Case studies for restored sites
- Methodologies for environmental risk assessment

Meetings of Experts

Article 31 Working Group on Relocation

Chilton (UK), 7 - 8 January 1993
6 participants from 3 countries and the Commission

Principal subjects:

- Criteria for relocation
- Generic guidance on intervention levels

ECURIE - Experts

Brussels (B), 24 February 1993
14 participants from 12 countries and the Commission

Principal subject:

- preparation of annual full-scale exercise, 1993

ECURIE - Experts

Luxembourg (L), 5 - 7 April 1993
14 participants from 12 countries and the Commission

Principal subject:

- examination of results of annual full-scale exercise, 1993

Third meeting of the Advisory Committee established by Article 19 of Council Directive 92/3/EURATOM on the supervision and control of shipments of radioactive waste between Member States and into and out of the Community.

Luxembourg (L), 15 - 16 April 1993

18 participants from 10 countries and the Commission

Principal subject:

- Standard documents referred to in the Directive.

Fourth meeting of the Advisory Committee established by Article 19 of Council Directive 92/3/EURATOM on the supervision and control of shipments of radioactive waste between Member States and into and out of the Community.

Luxembourg (L), 3 May 1993

14 participants from 10 countries and the Commission

Principal subject:

- Standard documents referred to in the Directive.

Qualified experts in radiation protection.

Luxembourg (L), 20 July 1993

14 participants from 11 countries and the Commission

Principal subject:

- Community harmonisation of qualification criterias.

V

ERPET

EUROPÄISCHE AUS- UND FORTBILDUNG

AUF DEM GEBIET DES STRAHLENSCHUTZES

EUROPEAN RADIATION PROTECTION EDUCATION AND TRAINING

ENSEIGNEMENT ET FORMATION EUROPEENS

EN RADIOPROTECTION

V. ERPET - European Radiation Protection Education and Training

The CEC is promoting education and training activities in radiation protection in order to maintain and extend Community expertise in radiation protection, in particular in view of the forthcoming developments in the Community. These education and training activities are in compliance with Article 33 of the EURATOM Treaty.

Within the framework of ERPET (European Radiation Protection Education and Training), activities are organised by the Commission's services in charge of Radiation Protection: DG XI, DG XII and, where appropriate, the service for EURO Courses of the JRC-ISPRA, together with competent institutions in the Member States, existing cooperative groups or other groups created for this purpose.

The education and training activities involve:

- Organisation of training courses;
- Development and provision of information and training packages;
- Exchange of scientists and promotion of participation in scientific conferences.

On the following pages, training courses and other activities organised in the period from 1 December 1992 - 31 October 1993 are listed. The courses provided coordinated, up-to-date programmes on key problems in radiation protection, for which a consistent Community approach is crucial. Some of these courses will be repeated after evaluation and updating in order to ensure that a larger number of interested persons become acquainted with the most advanced knowledge in radiation protection.

A uniform evaluation by means of questionnaires has been introduced. The compiled comments on organisation, course content and lecturers' performance contributed to the improvement of these three aspects of the ERPET Action.

☞ For full names of acronyms and abbreviatons please see chapter VII.

ERPET CEC Course on Current Techniques in Radiation Mutagenesis

Leiden (NL), 4 December 1992
Organizers of the course and 11 students from CEC countries

Principal subjects:

- Molecular analysis of mutants;
- Gene specific repair;
- Premature chromosome condensation;
- In situ hybridisation.

Training Course on Quality Assurance and Radiation Protection in Diagnostic Radiology

Jointly organised by the CEC, Unitá Sanitaria Locale N° 7, Udine (I) and IRS Ltd. Liverpool (UK), course held in English and Spanish.

Udine (I), 31 May - 4 June 1993

65 participants from all regions of Italy

Purpose: to provide up-to-date operational aspects and measures of radiation protection practice in diagnostic radiology.

Principal subjects:

- Background information to operational and legislative framework for radiation protection of the patient as well as the staff
- Quality assurance in diagnostic radiology: quality requirements with regard to radiographic images and patient dose
- Quality control programmes: organisation, implementation, measurements
- Role of technical developments, practical demonstrations.

Target groups:

All those actively involved in the day-to-day practice of diagnostic radiology, as well as those responsible for education and training of radiographers and radiological technicians.

4th ERPET Training Course on Off-Site Emergency Planning and Response for Nuclear Accidents

Jointly organised by the CEC and CEN/SCK Mol (B)
Mol (B), 21 - 25 June 1993
57 participants from 23 countries

Principal subjects:

- Principles of intervention planing, organisation and decision making with respect to off-site intervention in the case of an accidental release of radioactive material to the environment
- Consequences of accidental releases
- Exposure pathways and health effects
- Agricultural countermeasures
- Environmental monitoring
- Emergency exercises

Tarjet groups:

Professionals involved in emergency planing.

Training Course on the use of the PC version of the Probabilistic Accident Consequence Code COSYMA

Jointly organised by the CEC, KfK Karlsruhe (D) and the NRPB, Chilton (UK)
Wallingford, Oxfordshire (UK), 28 June - 2 July 1993
37 participants from 20 countries

Purpose: Training in the use of PC COSYMA

Principal subjects:

- Training course on PC COSYMA
- Probabilistic accident consequence models
- Atmospheric dispersion and deposition
- Transfer the terestrial environment
- Health effects
- Use of software and preparation of input data for code

Tarjet groups:

Those profesionally involved with risk assessment in nuclear safety.

Second Summer School on Radiecology

Jointly organised by the CEC, IUR, Hungarian National Research Institute for Radiobiology and Radiohygiene.
Budapest (H), 26 July - 7 August 1993

25 participants from 11 countries and the Commission

Purpose: To fulfil the needs of qualified graduates in environmental radioactivity in the following fields:

- Basic principles applied to radiecology.
- Radionuclides transfer in terrestrial ecosystems, in aquatic ecosystems, in food chains and modelling.
- Ecological effects of radiation and its consequences.
- Dose limits and legislation.
- Accident situations and emergency procedures en relation to dose assessment and remedial actions.
- Application of radiological knowledge to waste management problems.

Target group:

Young scientists working at research centres, universities and government bodies, as well as in private industry involved in radioecology.

Training Course on the use of Decision Aiding Software for Optimising Radiological Protection

Jointly organised by the CEC, University of Leeds (UK) and TNO, Rijswijk (NL)
Leeds (UK), 6 - 8 September 1993
13 participants from 4 countries

Purpose: Training in the use of more complex optimisation programmes

Principal subjects:

- Setting of Multi-Attribute Value Theory
- Multi-Attribute Value Analysis
- Understanding Sensitivity Analysis
- Structuring Attribute Trees
- MAVT Exercises
- Uncertainty modelling

Target groups:
Those professionally involved with optimisation of radiological protection in nuclear safety and waste management

5th ERPET Training Course on Off-Site Emergency Planning and Response for Nuclear Accidents

Jointly organised by the CEC, Greek Atomic Energy Commission, Athens (GR) and CEN/SCK Mol (B)
Chalkida (GR), 25 - 29 October 1993

25 participants from 4 countries

Training in off-site emergency planning and response to nuclear accidents.

Principal Subjects:

- On-site emergency planning at Kozloduy plant
- Off-site emergency planning in Bulgaria
- Greek nuclear emergency plan and environmental monitoring
- Assessment of off-site consequences and real-time consequence assessment
- Consequences of accidental releases
- Exposure pathways and health effects
- Principles of intervention
- Agricultural countermeasures
- Environmental monitoring
- Emergency response exercises

Target groups:

Those involved in contingency planning e.g. civil protection and environmental protection officers, persons responsible for the management of radiation protection or emergency planning at nuclear facilities.

VI

AUSWAHL EINIGER AUF VERANLASSUNG DER KOMMISSION ERSCHIENENER VERÖFFENTLICHUNGEN

SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE OF THE COMMISSION

CHOIX DE PUBLICATIONS EDITEES SUR L'INITIATIVE DE LA COMMISSION

VI. Publications

The scientific research results of the Commission's Radiation Protection Actions are presented in articles published in scientific journals. References to these are given in the corresponding Progress Reports. In certain cases the Commission initiated surveys of detailed results of specific activities in the field of radiation protection and published them as monographs or proceedings. Short descriptions of those publications, published or prepared in the period from 1 December 1992 - 31 October 1993, are given on the following pages.

☞ For full names of acronyms and abbreviatons please see chapter VII.

VI MONOGRAPHS

International Chernobyl Project - Input from the Commission of the European Communities to the Evaluation of the Relocation Policy adopted by the former Soviet Union

Report prepared by J. Lochard and T. Schneider, CEPN (F) (Part A) and S. French, University of Leeds (UK) (Part B) under contract nos. BI7-0060-GB and 90-ET-027 for the Commission of the European Communities

Edited by: G.N. Kelly and F. Luykx

An International Advisory Committee was convened by IAEA at the request of the Government of the former USSR, to assess the concept used to enable the population to live safely in areas affected by radioactive contamination following the Chernobyl accident. The Commission was represented in the International Advisory Committee and this report summarises the major technical inputs it made to the evaluation. In particular an analysis was made of the costs and effectiveness of the existing and alternative policies to ensure safe living conditions and a series of Decision Conferences were held to identify the major factors that were influencing policy decisions in this area.

Report EUR 14543 EN, 1992, 151 pages

To be ordered through:

Office for Official Publications of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 15

Thyroid Cancer in Children living near Chernobyl

Expert Panel report on the consequences of the Chernobyl accident

Edited by D. Williams, A. Pinchera, A. Karaoglou, K.H. Chadwick

In January 1992, under the Radiation Protection Research Action, a Panel of experts was set up to evaluate the current situation concerning reported increased incidence of thyroid cancer in children living near Chernobyl at the time of the nuclear reactor accident on 26 April 1986.

The report written by this Panel documents their findings with respect to the occurrence of childhood thyroid cancer in Belarus and the Northern Ukraine. The Panel arrives to a consensus opinion and makes strong recommendations for urgent technical and humanitarian assistance and research cooperation.

Published by: The Commission of the European Communities - Radiation Protection Research and Training Programme

Report EUR 15248 EN, 1993, 108 pages
ISBN 92-826-5515-6

To be ordered through:

Office for official publications of the European Communities
Boîte Postale 1003
L-2985 Luxembourg

Price: ECU 13.50

Development of a general guideline for the radiological optimisation of the restoration of former nuclear sites.

Report prepared by Th. Zeevaert and P. Govaerts, SCK-CEN, Mol (B) for the European Commission

A methodology was developed for assessing dose impacts to man in normal evolution and accidental scenarios for various restoration approaches.

Distinction is made between primary sources (independent sources present at the time of abandonment of the nuclear activities) and secondary sources (formed or fed by others through the transplantation of radionuclides). The modelling steps comprise:

- the evolution of primary sources with time;
- the transport of radionuclides subsequent to their release, giving rise to secondary sources;
- the influence of barriers against release and transport, etc.;
- the dose impact assessments following various exposure pathways.

This dose assessment approach is illustrated by a simple example with a typical primary source (a contaminated soil layer) feeding a typical secondary source (a groundwater well) with the transport between the sources being counteracted by a vertical barrier.

Radiation Protection Series No. 59 EN, 1993, 77 pages.

To be ordered through:

European Commission
Radiation Protection Division
Mr. Herbert LELLIG
Centre Wagner C 336
Rue Alcide de Gasperi
L-2920 Luxembourg

Principles and methods for establishing concentrations and quantities (exemption values) below which reporting is not required in the European Directive.

Report prepared by M. Harvey, S. Mobbs, J. Cooper, A.M. Chapuis, A. Sugier, T. Schneider, J. Lochard and A. Janssens.

The Community Basic Safety Standards for the health protection of the general public and workers against the dangers of ionising radiation incorporate values of activities not to be exceeded so that the requirements for reporting and obtaining prior authorisation of activities involving a hazard arising from ionising radiation need not be applied (Article 4 of Council Directive 80/386). For this purpose all relevant nuclides have been classified in four groups, according to their relative toxicity. Also radioactive substances of a concentration less than 100 Bq/g are exempted from this requirement, this limit being increased to 500 Bq/g for solid natural radioactive substances. These exemptions, while allowing competent authorities in Member States to disregard a multitude of trivial practices, have so far not given rise to any situations where the health of the general public or of workers were put at risk. However, the opportunity of a major revision of the Basic Safety Standards, to bring these in line with the latest recommendations of the ICRP (Publication 60), was taken to introduce a more transparent and consistent methodology for establishing exemption levels on a nuclide-specific basis.

Radiation Protection Series No. 65 EN, 1993, 94 pages.

To be ordered through:

European Commission
Radiation Protection Division
Mr. Herbert LELLIG
Centre Wagner C 336
Rue Alcide de Gasperi
L-2920 Luxembourg

Radiological Protection Principles for relocation and return of people in the event of accidental releases of radioactive material.

Recommendations of the group of experts set up under the terms of Article 31 of the Euratom Treaty.

The report sets out the principles for intervention and elaborates upon their application to the relocation of people after and accidental release of radioactive material. Because of the diversity of circumstances that might by euncountered followoing an accident, there are limits to the degree to which detailed planning for relocation can be made in advance or, indeed, would be sensible. The guidance given here has, therefore, been kept deliberately simple and is intended to be broadly applicable. Of necessity it will need to be refined in the light of the particular circumstances of an accident.

The guidance is intended to form a basic framework for decision making within which other factors and considerations can be integrated. Indeed, social and political factors which go beyond the scope of this guidance will need to be taken into account in the event of an accident, and decisions will ultimately be taken at political level.

Radiation Protection Series No. 64, 1993, 42 pages.

To be ordered through:

European Commission
Radiation Protection Division
Mr. Herbert LELLIG
Centre Wagner C 336
Rue Alcide de Gasperi
L-2920 Luxembourg

European Guidelines for Quality Assurance in Mammography Screening

Report prepared by: A. Kirkpatrick, S. Törnberg, M.A.O. Thijssen, in collaboration with the CEC Study Group on Quality Control in Mammography

Edited by C.J.M. de Wolf

This report outlines the requirements of a Quality Assurance programme with regard to a number of aspects of the mammographic screening system. It does not attempt to define the guidelines for treatment. The report gives detailed guidance for the medical diagnostic and technical aspects of the screening test itself. The document is merged from two documents: the general Quality Assurance guidelines which have been provided by the CEC "Europe against Cancer" Programme, and the European Protocol for the Quality Control of the Technical Aspects of Mammography Screening which has been prepared within the framework of the CEC Radiation Protection Actions.

With regard to the quality of mammography screening, three aspects are of prime importance: medical performance, organisation and the imaging process.

The Guidelines deal with these three aspects and incorporate the present state of knowledge on systematic breast cancer screening. They are applicable to all breast screening units and mammography equipment within all medical services. The European Protocol for the Quality Control of the Technical Aspects of Mammography Screening includes 12 sample data sheets for quality control reporting.

The list of references indicates nearly all existing quality control guidance available at local, regional and national level.

These Guidelines are available in the following languages: English (original), Danish, French, German, Greek, Italian, Portuguese and Spanish.

Published by the Commission of the European Communities in the Medicine and Health series.

EUR 14821 EN, 1993, 86 pages.
ISBN 92-826-5644-6

To be ordered through
Office for Official Publications
of the European Communities
Rue Mercier, 2
L-2985 Luxembourg

Price: ECU 7.50

PROCEEDINGS

The Natural Radiation Environment

Proceedings of a meeting jointly organised by the United States Department of Energy, the International Atomic Energy Agency, the University of Salzburg and the CEC.

Salzburg (Austria), 22 - 28 September 1991

Edited by A. Janssens, W. Lowder, M. Olast, J. Sinnaeve and F. Steinhäusler

These regularly organised international symposia always generate a lot of interest because of the rapid progress of knowledge and new development in this field.

In the opening session it was clearly stated that the radon issue is one of the major problems in radiation protection and underlined the need for international collaboration in order to optimise the research effort in different countries.

One can refer to the latest UNSCEAR report and to the requests for technical assistance from more than fifty-five countries to the IAEA assistance, to illustrate the seriousness of the radon problem and its regulatory implications.

In the course of the symposium, it was also emphasised that there is an urgent need to develop research to reduce the uncertainties associated with our present knowledge about the health impact of this exposure.

One can hope that the increase of data coming from measurements of radon in the human environment, from experimental investigations and from epidemiological studies will lead to a realistic assessment of the health impact, which has the approval of the whole scientific community.

This NRE V symposium fortunately was not only a radon symposium, many of other important subjects, such as the radiological impact of non-nuclear industrial releases, and the exposure of the public and of workers to natural sources of radiation in non-domestic environments, were also addressed in this symposium.

Published by Nuclear Technology Publishing, Vol. 45 Nos. 1-4 (Supplement)
EUR 13892 EN, 1992, 900 pages
ISBN 1 870965 140

To be ordered through:
Nuclear Technology Publishing
P.O. Box 7
GB-TN23 1YW Ashford, Kent

Price £.Stg. 150

Test Phantoms and Optimisation in Diagnostic Radiology and Nuclear Medicine

Proceedings of a Discussion-Workshop jointly organised by the CEC, GSF, Neuherberg, ICRU and EFOMP
Würzburg (D), 15 - 17 June 1992
Edited by B.M. Moores, N. Petoussi, H. Schibilla and D. Teunen

The workshop was perceived as a forum for discussions of test objects and phantoms, computational models and optimisation in diagnostic radiology and nuclear medicine. The current status of this field was presented by invited overview papers backed up by proffered papers in the form of posters and by discussions. They concentrated on requirements of design, implementation and the relevance of test objects, phantoms and computational models most suitable for quality control under different physical and clinically realistic conditions. The contributions should help to establish guidance for more standardisation of design and use of test objects, phantoms and computational models as well as the evaluation of the obtained data.

Nineteen overview papers and 60 proffered papers are reproduced, together with the summarised discussions and comments. They reflect the concepts and perspectives of some 170 participants coming from 28 countries.

The Proceedings are subdivided into 9 chapters:

1. Requirements for Test Objects and Phantoms
2. Applications in Conventional Radiology and Fluoroscopy
3. Mammography
4. Conventional and Computed Tomography
5. Digital Radiography
6. Nuclear Medicine
7. Computational Models
8. ICRU - activities in Phantom Design and Application
9. Conclusions

In Chapter 8, 25 phantom specific sheets are published which complement the ICRU Report, 48 on Phantoms and Computational Models in Therapy, Diagnosis and Protection.

Published by Nuclear Technology Publishing, Vol. 49 Nos. 1-3
EUR 14767 EN, 1993, 416 pages.
ISBN 1 870965 26 4

To be ordered through:
Nuclear Technology Publishing
P.O. Box No. 7
GB-TN23 1YW Ashford, Kent

Price: £.Stg. 80.00

Radiation exposure of civil aircrew.

Proceedings of a workshop organised by the European Commission
Luxembourg (L), 25 - 27 June 1991
Edited by G. Reitz, K. Schnuer and K. Shaw

Cosmic radiation generates secondary high energy radiation in the outer layers of the atmosphere. Only a part of it reaches the surface on the earth and contributes to the natural radiation exposure of the general public.

The level of this secondary and cosmic radiation depends to some extent on the geographical position, but essentially on the altitude above the ground level; the maximum radiation level occurs at about 20.000 meters. Since the advent of supersonic flights and space travel, scientists in the field of radiation protection have been confronted with the problem of cosmic radiation and its impact on aircrew. In recognition of these effects and the circumstance that modern jet aircraft are operating at high altitudes, the question arises as to whether aircrew have to be considered as occupationally exposed workers. These are, by definition (Basic Safety Standards for the Health Protection of the General Public and Workers Against the Danger of Ionising Radiation, 15 July 1980), those persons subjected as a result of their work, to an exposure liable to result in annual doses exceeding one-tenth of the annual dose limits laid down for workers.

The 1990 recommendations of the International Commission on Radiological Protection state that there should be a requirement to include exposures to natural sources during flight in jet aircraft as part of occupational exposure for aircrew. The radiation exposure of aircrew is an important topic and this Workshop brought together international experts to provide information and discussion. The Workshop was organised in a relatively short timescale and not all of the papers were available in final form at the meeting. Authors were subsequently allowed time to finalise their contributions and their papers were then subjected to a lengthy review procedure.

The contributions of this Workshop provided the latest scientific information for those active in the field of radiation protection, in aviation companies and industry as well as in regulatory organisations. They initiated objective discussions of the subject and helped to dispel some of the misunderstandings. The roundtable discussion gave some guidelines on future measures to be taken by European Community together with the institutions or organisations active in the field.

Published by Nuclear Technology Publishing (UK), Vol. 48 No. 1
EUR 14964 EN, 1993, 145 pages

To be ordered through:

Nuclear Technology Publishing
P.O. Box No. 7
GB-TN23 1YW Ashford, Kent
Price: £Stg. 30

Relative Effectiveness of Agricultural Countermeasure Techniques: REACT

Proceedings of a meeting organised by the CEC.

Brussels (B), 1 - 4 October 1991

Edited by B.J. Howard and G. Desmet

The Radiation Protection Research Action of DGXII of the EC together with the Member States, having initiated numerous research activities in this field has considered it necessary to reach a comprehensive view with respect to the most appropriate actions which need to be taken after a nuclear accident.

This proceedings are the result of a workshop meeting were the efficiency, feasibility and realistic applicability of each countermeasure was discussed and evaluated.

Published by the Science of the Total Environment, Vol. 137 Nos. 1-3, Amsterdam, EUR 14508, 1993, 320 pages.

To be ordered through:
Elsevier Science Publishers, Journal Department,
P.O. Box 211
NL-1000 AE Amsterdam

Price: Dfl. 238

Neutron Dosimetry

Proceedings of the 7th Symposium on Neutron Dosimetry jointly organised by Physikalisch-Technische Bundesanstalt (PTB), US Department of Energy and the Commission of the European Communities.

Berlin (D), 14 - 18 October 1991

For physical and biological reasons, neutron dosimetry poses more complex and more technical and conceptual problems than photon dosimetry.

Recently, the International Commission on Radiological Protection (ICRP), in its publication 60, has put forward an alternative concept for risk related quantities, based on radiation weighting factors, and uses the quantity effective dose, E, instead of effective dose equivalent, H_E. The consequence of these new recommendations was discussed extensively and controversially during the Symposium.

The ICRP recommendations to reduce exposure limits and to increase the quality factor for neutrons also have implications on practical neutron monitoring. One of the important tasks for immediate neutron dosimetry research is therefore to develop dosemeters with considerably lower detection limits.

The Symposium revealed a continued increase in attention given to neutrons of high energies. In radiation protection, high energy neutron exposures occur near accelerators, during jet aircraft flights and during space missions. In tumour therapy with fast neutrons higher neutron energies (up to 70 MeV) are used to improve the dose distribution depth. At these energies the implications of the new ICRP recommendations for neutron and mixed field exposures including protons in addition to gamma rays require further detailed studies.

The Proceedings present a comprehensive compilation of results from ongoing research in neutron dosimetry.

Published by Nuclear Technology Publishing in Radiation Protection Dosimetry Vol. 44, Nos. 1-4.

EUR 14547 EN, 1992, 470 pages.
ISBN 1 870965 16 7

To be ordered through:
Nuclear Technology Publishing
P.O. Box No. 7
GB- TN23 1YW Ashford, Kent

Price: £ Stg. 80.00

Decision Making for Off-site Emergency Management

Proceedings of a Workshop held at Schloss Elmau and Co-organised by the Commission of the European Communities in collaboration with Office of Health and Environmental Research United States Department of Energy and GSF, Forschungszentrum für Umwelt und Gesundheit GmbH, Germany.

Schloss Elmau, Bavaria, 25 - 30 October 1992

Edited by: G.N. Kelly and G. Fraser

Considerable progress has been made in the development of decision support systems for off-site emergency management in the past decade, in particular following the Chernobyl accident. The proceedings of this workshop summarize the progress made in many countries together with plans for the future.

Notwithstanding the progress made, much yet remains to be done to ensure that best use will be made of available information in the off-site management of any future accident. Areas where additional effort is needed are identified and include the need for improved communication with decision makers, the treatment of uncertainties, exchange of monitoring data between countries and making best use of potentially conflicting model predictions and measurements.

Published by NTP, Ashford, Kent

Report EUR 15043 EN, 1993, 378 pages
ISBN 1 870965 25 6

To be ordered through:
Nuclear Technology Publishing
P.O.Box No. 7
GB-TN23 1YW Ashford, Kent

Price: £ Stg. 60

What's New and What's Needed in Radiation Effects Research

Proceedings of the second Bad Honnef contractors meeting held in Bad Honnef (D)

Bad Honnef (D), 29 March - 1 April 1993

The meeting brought together physicists, radiation chemists, researchers working in molecular and cellular radiobiology and in animal research together, as well as epidemiologists. The necessity for multidisciplinary approach in research aimed at understanding the mechanisms of radiation induced changes in animals and humans is a major point of the report. It also identifies what is new and what is needed in radiation effects research for the fourth framework programme.

To be ordered through:

The Commission of the European Communities
Radiation Protection Research - DG XII.F.6
Rue de la Loi 200
B-1049 Brussels

VII

LISTE DES ACRONYME UND ABKÜRZUNGEN

LIST OF ACRONYMS AND ABBREVIATIONS

LISTE DES ACRONYMES ET DES ABREVIATIONS

AECB	Atomic Energy Control Board, Ottawa (Canada)
AECL	Atomic Energy of Canada Limited (Canada)
AFPPE	Association Française du Personnel Paramédical d'Electrocardiologie, Paris (F)
AIRM	Associazione Italiana di Radioprotezione Medice (I)
AIRP	Associazione Italiana di Protezione contro le Radiazioni (I)
ALARA	As Low As Reasonably Achievable
CAATS/INSERM	Centre d'évaluation pour l'Assurance de qualité des Applications Technologiques dans le domaine de la Santé/Institut National de la Santé et de la Recherche Médicale, Cachan (F)
CEA	Commissariat à l'Energie Atomique, Fontenay-aux-Roses (F)
CEC	Commission of the European Communities, Brussels (B)
CEDHYS	Centre de Développement des Etudes et Applications en Hygiène et Sécurité, Paris (F)
CEN/SCK	Centre d'Energie Nucléaire/Studie Centrum voor Kernenergie, Mol (B)
CEPN	Centre d'étude sur l'Evaluation de la Protection dans le domaine Nucléaire, Fontenay-aux-Roses (F)
CIEMAT	Centro de Investigaciones Energéticas, Medioambientales y Technológicas, Madrid (E)
CIR	Centre International de Radiopathologie, Fontenay-aux-Roses (F)
CNEN	Comitate Nazionale per la Ricerca e per lo Sviluppo dell'Energia Nucleare e delle Egnergie Alternative, Rome (I)
COSYMA	Code System Maria
CRSA	Centro Regionale per la Sperimentazione Agraria per il Friuli-Venezia-Guilia, Udine (I)
EAR	European Association of Radiology
EBMT	European Bone Marrow Transplant Group
ECP()	Experimental Collaboration Project (No.)
ECURIE	Early Exchange of Information in the Event of Radiological Emergency
EFOMP	European Federation of Organisations of Medical Physics, York (UK)
ENEA/DISP	Comitato Nazionale per la Ricerca e per lo Sviluppo dell'Energia Nucleare e delle Energie Alternative, Direzione Sicurezza Nucleare e Protezione Sanitaria, Rome (I)
ENEL	Ente Nazional per l'Energia Elletrica, Roma (I)
ERPET	European Radiation Protection Education and Training CEC DG XI/A/1, Luxembourg (L) & DG XII/D/3, Brussels (B)
EULEP	European Late Effects Project Group
EURADOS/CENDOS	European Radiation Dosimetry Group/Collection and Evaluation of Neutron Dosimetry Data
EUROMET	European Metrology
DG	Directorate General of the CEC
GSF	Gesellschaft für Strahlen- und Umweltforschung, Neuherberg (D)
IAEA	International Atomic Energy Agency (A)
IARC	International Agency for Research on Cancer, Lyon (F)
ICRP	International Commission on Radiological Protection
ICRU	International Commission on Radiation Units and Measurements
ICSTM	Imperial College of Techn. and Medicine Science, London (UK)
IFE	Institute of Freshwater Ecology, Ambleside (UK)
INTECHMER-CNAM	Institut National des Techniques de la Mer - Conservatoire National des Arts et Métiers-Cherbourg (F)

INSTN	Institut National des Scienes et Techniques Nucléaires, Saclay (F)
IOMP	International Organisation of Medical Physicists
IRS	Integrated Radiological Services Ltd, Liverpool (UK)
ISH	Institut für Strahlenhygiene/Bundesamt für Strahlenschutz, Salzgitter/Neuherberg (D)
ITE	Institute of Terrestrial Ecology, Grange-over-Sands (UK)
ITRI	Inhalation Toxicology Research Institute, Albuquerque, NM (USA)
ITRI/TNO	Instituut voor Toegepaste Radiobiologie en Immunologie, TNO Rijswijk (NL)
IUR	International Union of Radioecologists
JRC	Joint Research Center of the CEC at ISPRA
JSP()	Joint Study Project (No.)
KFA	Forschungsanlage, Jülich (D)
KfK	Kernforschungszentrum Karlsruhe (D)
KUL	Katholieke Universiteit Leuven (B)
LNETI	Laboratorio Nacional de Engenharia e Tecnologia Industrial, Lisboa (P)
MAFF	Ministry of Agriculture, Food and Fisheries, Lowestoft (UK)
MARIA	Methods for Assessing the Radiological Impact of Accidents
MLURI	McAulay Land Use Research Institute, Edinburgh (UK)
NEB	Nuclear Energy Board of Ireland, Dublin (IRL)
NIRP	National Institute of Radiation Protection, Stockholm (S)
NCSR	Democritos, National Centre for Scientific Research, Athens (GR)
NRPB	National Radiological Protection Board, Chilton (UK)
OECD/NEA	Organisation for Economic Cooperation and Development/Nuclear Energy Agency
ORNL	Oak Ridge National Laboratory, Knoxville, Tennessee (USA)
PTB	Physikalisch-Technische Bundesanstalt, Braunschweig (D)
RADE-AID	Radiological Accident Decision Aiding System
RBE	Relative Biological Effectiveness
REM	Radioactivity Environmental Monitoring
RERF	Radiation Effects Research Foundation, Hiroshima (Japan)
RIVM	Rijks Instituut voor Volksgezondheid en Milieu, Bilthoven (NL)
SCRPI	Service Central pour la Protection contre les Rayonnements Ionisants, Le Vésinet (F)
SEPR	Sociedad Española de Proteccion Radiologica (E)
SFEN	Société Française d'Energie Nucléaire, Paris (F)
SFRP	Sociedad Española de Proteccion Radiologica (E)
SRD	Safety and Reliability Directorate, Warrington (UK)
SUAS	Swedish University of Agricultural Sciences, Umea (S)
TEPC	Tissue-Equivalent Proportional Counter
TNO	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek, Rijswijk (NL)
UKAEA	United Kingdom Atomic Energy Authority, Harwell (UK)
ULB	Université Libre de Bruxelles, Brussels (B)
US DOE	US Department of Energy, Washington DC (USA)
US EPA	US Environmental Protection Agency
US NCI	US National Cancer Institute, Bethesda (USA)
US NRC	US Nuclear Regulatory Commission
US NIES	US National Institute of Environmental Sciences
USL	Unitá Sanitaria Locale, N° 7, Udine (I)
WHO	World Health Organisation

VIII

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(c) Risks and management of radiation protection.

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(ii) to prevent and counteract harmful effects of radiation;
(iii) to assess radiation risks and provide methods to cope with the consequences of radiation accidents.

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